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Special Study: Trichloroethylene Exposure in Women and Men

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13. ABSTRACT *(Maximum 200 words)*

The purpose of this study was to conduct controlled human trichloroethylene (TCE) inhalation studies to support physiologically-based pharmacokinetic (PB-PK) modeling of TCE exposure in healthy men and women. TCE is a volatile liquid used in degreasing operations, industrial cleaning, paint strippers, rug cleaners, spot removers, and typewriter correction fluid with widespread industrial and military use. Eight female and nine male subjects willing to provide informed consent were medically qualified for participation, then were exposed to 4 hours of 50 ppm or 100 ppm TCE at rest in a controlled environmental chamber. Following exposure, subjects remained in the chamber breathing clean air at rest and during sleep for an additional 18 hours. Periodically during and after exposure, blood samples were taken via venous catheter and urine samples were collected via specimen containers. The blood and urine samples were partitioned and treated with reagents, and the subsamples flash-frozen. Frozen samples were shipped to designated laboratories for chemical analysis, and the resulting TCE and TCE-metabolite data transmitted to the Tri-Service Toxicology group at Wright-Patterson Air Force Base (WPAFB) for PB-PK model analysis. Results of the TCE PB-PK model analyses are to be reported elsewhere by the WPAFB investigators.

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Paul A. Kyle 2/1/96

PI - Signature Date

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SYMBOLS AND DEFINITIONS

ρ	Resistivity of blood (ohm-cm)
Ω	Ohms, unit of electrical resistance
ACCEL	ICG acceleration (cardiac contractility) (Ω/s^2)
cfm	Cubic feet per minute
CO	Cardiac output
DCA	Dichloroacetic acid
dZ/dt	ICG first derivative
dZ/dt_{peak}	Peak systolic amplitude of dZ/dt (Ω/s)
ECG	Electrocardiogram
EPA	Environmental Protection Agency
ft^3/min	Cubic feet per minute
HEGA	High efficiency gas filters
HEPA	High efficiency particle filters
Hz	Hertz
ICG	Impedance cardiogram
IL	Instrumentation Laboratories
L	Distance between ICG electrodes
l/min	Liters per minute
LA	Left arm
LL	Left leg
mm	Millimeters (of ST segment depression)
mV	Millivolts
N	Number of Subjects
PB-PK	Physiologically-based pharmacokinetic
ppm	Parts per million
QC	cardiac output
QP	ventilation
RA	Right arm
RATE	heart rate (beats/min)
RL	Right leg
RTI	Research Triangle Institute
SV.	Stroke volume
TCE	Trichloroethylene
TEI	Thoracic Electrical Impedance
TCA	Trichloroacetic acid

SYMBOLS AND DEFINITIONS (continued)

TCOH	Trichloroethanol
TZavo	Time from ECG Q-wave to aortic valve opening (ms)
TZpeak	Time from ECG Q wave to dZ/dt_{peak} (ms)
TZx	Time from ECG Q-wave to aortic valve closing (ms)
V_5	Placement of chest electrode at precordial lead 5
WPAFB	Wright-Patterson Air Force Base
WRAIR	Walter Reed Army Institute of Research
ΔZ	Cardiac bioimpedance signal
Zo	Mean thoracic electrical impedance

1.0 INTRODUCTION

1.1 Nature of the Problem

1,1,2-Trichloroethylene (TCE) (CAS #79-01-6) is a volatile liquid used in degreasing operations, industrial cleaning, paint strippers, rug cleaners, spot removers, and typewriter correction fluid. TCE has been used as an anesthetic agent, and chloral hydrate, a TCE metabolite, is commonly used as a sedative in adults, children, and infants (Campus-Outcalt, 1992). Widespread industrial usage and poor waste management has led to widespread environmental contamination (Abelson, 1990). TCE has been detected in over one-third of hazardous waste sites and 10% of groundwater sources.

TCE has been and continues to be used extensively in industrial and military processes as a degreasing agent, so direct exposure of male and female maintenance personnel involved in these activities may be substantial. Because of both its widespread industrial and military use and the consequences of inadequate disposal methods, TCE is now a priority ground water contaminant for the Department of Defense (DoD) and the Environmental Protection Agency (EPA). DoD remediation of TCE at contaminated sites to the EPA regulation of 5 ppb in drinking water has been expensive, although these cleanup standards may be unnecessarily conservative. Risk assessment using a human-validated, physiologically-based pharmacokinetic (PB-PK) model might establish that current environmental cleanup standards may be safely lowered with no increased risk to sensitive human populations.

1.2 Background of Previous Work

The toxicology of TCE and its metabolites [dichloroacetic acid (DCA), trichloroacetic acid (TCA), trichloroethanol (TCOH)] is controversial. Acute workplace exposure causes neurologic, cardiac, respiratory, and hepatic effects (Clayton and Clanton, 1981; Kostrzewski et al., 1993). Oral TCE administration has been shown to be carcinogenic in B6C3F1 mice, but not in Osborne-Medel, Fischer 344, or Sprague-Dawley rats. The carcinogenic effect is thought to be linked more with DCA and TCA than with TCE (Herren-Freund et al., 1987; Bull et al., 1990; Larson and Bull, 1992; Templin, 1993). DCA has not yet been measured in humans, and there is some question whether it is even produced. An epidemiological analysis of 14,457 occupationally-exposed humans showed no increased risk of cancer (Spirtas et al., 1991), and no study conclusively demonstrates that TCE causes cancer in humans (Axelson et al., 1994).

Regulatory and scientific agency opinions on TCE exposure are varied:

- TCE is listed as a hazardous substance in the Clean Air Act of 1990 based upon induced liver cancers in laboratory animals. Currently, the US Environmental Protection Agency (EPA) has withdrawn all cancer potency estimates pertaining to TCE (EPA 1994a,b).

- The Occupational Safety and Health Administration does not classify TCE as a human carcinogen. The Occupational Safety and Health Administration has set a permissible airborne-exposure level of 100 ppm for an 8-hour average occupational exposure

- The American Conference of Governmental Industrial Hygienists has set an 8-hour time weighted average exposure limit of 50 ppm, with a short-term exposure limit of 100 ppm permitted for 15 minutes up to 4 times a day. The American Conference of Governmental Industrial Hygienists classifies TCE as type A5 "Not Suspected as a Human Carcinogen."

- The National Institute of Occupational Safety and Health gives a recommended exposure limit of 25 ppm for TCE. NIOSH classifies TCE as an occupational carcinogen.

- The International Agency for Research on Cancer places TCE in Group 3, not classified as to its carcinogenicity in humans.

- The Federal Republic of Germany 1989 Maximum Concentration values in the Workplace classifies TCE in Group B, justifiably suspect of having carcinogenic potential.

The magnitude of TCE contamination and potential cost of remediation makes it imperative that scientifically-supportable risk-benefit analyses are performed to determine an appropriate course of action for cleanup (Abelson, 1990). PB-PK models of TCE uptake, residence, metabolism, and elimination are analytical tools that can be used for assessing risk (Fisher and Allen, 1993).

The PB-PK model of TCE/TCA developed by Allen and Fisher (1993) consists of four compartments, each identified with specific groups of tissues, plus a mechanism for exchange of TCE between the pulmonary blood and the end-alveolar air (Figure 1). Metabolism was assumed to be only in the liver compartment. Initially, both a saturable pathway and a purely first-order pathway were considered. No other means of TCE elimination (e.g., breast milk, urine) were included. A fraction of the TCE metabolized by the saturable pathway was enzymatically converted to TCA. TCA was assumed to be distributed in a fixed-volume compartment and to be cleared by a first-order process.

PB-PK models are being developed for animals and humans, and *in vivo* model-validation studies have been done in mice and rats (Fisher et al., 1991; Dallas et al., 1991), including TCE transfer in breast milk to nursing pups (Fisher et al., 1990). Although gender-specific models for benzene exposure have been investigated (Table - Brown, 1994), human PB-PK models for TCE have been reported only in human males (Monster et al., 1976; Sato et al., 1977). We are unaware of any human model validation for TCE and multiple TCE metabolites (DCA, TCA, and TCOH) in both male and female blood, urine, breath, and breast milk.

Differences in individual physiology and activity level will cause TCE dose to vary significantly among equivalently exposed individuals. Because rate and equilibrium level of TCE uptake are strongly related to blood volume, body composition, cardiac output (QC), and ventilation (QP) (Figure 1), gender and non-gender differences such as body size, blood volume, lean/fat ratio, and activity level will produce differences in TCE uptake, metabolism, and elimination (Nomiyama, 1971; van Baak, 1990; Swartz et al., 1894; Somani, et al., 1990; O' Tool, 1989). PB-PK models for TCE should incorporate these parameters and be validated for a variety of individual characteristics and activities.

1.3 Purpose of the Present Work

The purpose of this study was to conduct controlled human TCE inhalation studies to support PB-PK modeling of TCE exposure in healthy men and women. The Tri-Service research effort in TCE PB-PK modeling is being led by Dr. Jeffrey W. Fisher, Senior Staff Scientist, Toxicology Division, Occupational and Environmental Health Directorate, Wright-Patterson Air Force Base (WPAFB). Dr. Fisher has developed PB-PK models for rats that include lactation and transfer of TCE and TCA to rat pups. Higher concentrations of TCA than TCE are transferred to breast-feeding rat pups. He has also extrapolated the rat-developed PB-PK model parameters for use in risk analysis in humans. The primary objective of the current study was to acquire biological samples and relevant experiment data under conditions of controlled TCE inhalation exposure, and to provide such samples and exposure data for analysis and PB-PK modeling at WPAFB-designated laboratories.

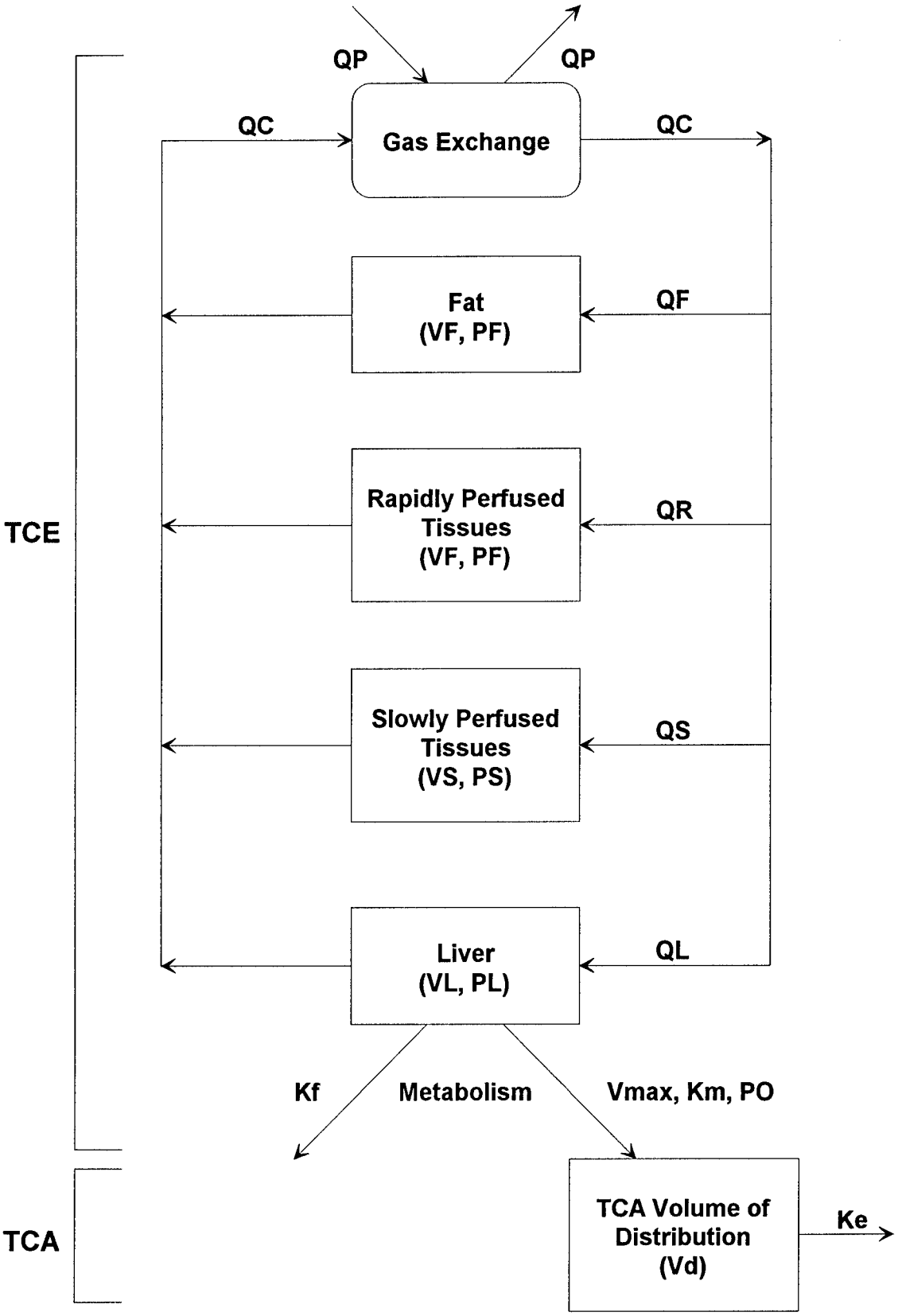

Figure 1. Schematic of TCE/TCA pharmacokinetic model (from Allen and Fisher, 1993).

The specific aims of the study were:

1) To design a TCE-inhalation protocol and obtain approval to perform such studies from the RTI Institutional Review Board and the U.S. Army Human Studies Research Review Board.

2) To recruit and expose a normal population of men and women to controlled atmospheres of clean air, 50 ppm TCE, and 100 ppm TCE at rest.

3) To provide physiological data, blood samples, and urine samples for further analysis at WPAFB and WPAFB-designated laboratories.

4) To provide quality assurance of TCE exposure concentrations.

5) To cooperate with EPA scientists in the acquisition of cognitive and sensory data for assessment of TCE effects on cognitive performance and short-term health effects.

1.4 Technical Approach

To meet the goals and objectives of this project, RTI performed the following tasks:

1) Reviewed the relevant scientific and regulatory literature on TCE health effects and PB-PK modeling.

2) Wrote a series of experiment protocols for human studies, submitted them to the RTI and U.S. Army human use review committees, revised them as necessary, and obtained approvals.

3) Prepared detailed descriptions of experiment procedures.

4) Modified the RTI Human Studies Facility exposure chamber for controlled exposure to TCE.

5) Acquired, prepared, calibrated, and tested all necessary laboratory instrumentation, data acquisition software, and analysis software.

6) Recruited subjects, medically qualified them, exposed them to TCE and gathered data according to the approved protocols.

7) Collected experiment data, blood samples, and urine samples and shipped them to WPAFB and WPAFB-designated laboratories for analysis.

2.0 METHODS, RESULTS, AND DISCUSSION

2.1 Subjects

The original study population goal was 20 healthy females and 5 healthy males, 18-35 years old, willing to provide informed consent and comply with the requirements of the experiment protocol. After a series of modifications to the experiment design, that included extending the monitoring period to 24 hours, the study population goal was changed to 10 female and 10 male subjects.

Criteria for exclusion were pregnancy, chronic use of alcohol, history of hepatic disease, cardiac conduction abnormalities, congenital cardiac defects, asthma, history of early parental cardiac disease, blood abnormalities, or other cardiopulmonary disorders.

Recruitment advertisements were designed, then submitted to the RTI IRB, to the RTI Office of Human Resources, and to the Surgeon General's Human Subjects Research Review Board (HSRRB) for approval after final approval of the experiment protocol. The advertisements were then posted in newspaper ads and on a regional Internet news group.

Individuals who responded to the advertisements were informed by phone of the project objectives and their potential role as a participant. If he/she was interested, a brief questionnaire was given over the phone for prequalification. This questionnaire included items such as gender, age, any known illness, etc. Responses to these questions were not recorded; a note was simply made whether the questions were completed. Individuals passing the prescreening were scheduled for medical screening.

Subject qualification took several steps. When subjects arrived for medical screening, informed consent was obtained by a direct discussion between the attending investigator (or the senior technician) and the volunteer. Subjects were informed about the experimental procedures, risks, and safeguards, and the purposes of the screening study before signing the Informed Consent. The subject read the consent form and was allowed to ask questions. If satisfied, the subject signed the consent form.

A medical history was taken for review of prior illness and familiar illness patterns. Subjects gave a family and personal medical history (with emphasis on cardiovascular, pulmonary, and hepatic systems) and received a physical examination that included blood analysis. Anthropometric measurements and a lean/fat ratio will be recorded. Routine blood work (SMA20) and hematological (CBC, differential) tests were performed on all subjects. Females had a serum pregnancy test before each TCE exposure. Subjects passing the medical exams were deemed fully qualified for the TCE exposure study.

2.2 Experiment Protocol

The focus of the experiment design was to expose healthy men and women to controlled TCE exposures, to collect biological samples, and to collect cardiac function data for TCE PB-PK modeling. Planned extensions to the core experiment were: (1) include a small number of lactating women to determine TCE and TCE-metabolite transfer to breast milk, and (2) cooperate with EPA Health Effects Research Laboratory (HERL) scientists for collection of cognitive performance data, symptom data, and expired breath samples. In the course of considering these extensions and responding to concerns from the RTI IRB and US Army HSRRB, a series of experiment protocols were developed, submitted for review, and revised as indicated in Table 1.

Table 1. Evolution of TCE Inhalation Protocols

	Proposed	July-94	April-95	May-95	Sep-95
Males	5	5	10	10	10
Non-Lactating Females	15	15	10	10	10
Lactating Females	5	5	5 (if possible)	0	0
Pre-exposure	15 min Air Exercise	15 min Air Rest	15 min Air Rest	15 min Air Rest	15 min Air Rest
Exposure	4 hours 50 ppm /100 ppm Rest & exercise	4 hours 50 ppm /100 ppm Rest	4 hours 50 ppm /100 ppm Rest	4 hours 50 ppm /100 ppm Rest	4 hours 50 ppm /100 ppm Rest
Post-exposure	2 hours	20 hours 2nd/4th day 2 week urine	20 hours 2/3/4th day	20 hours 1/2/3/4th day 2 week urine	20 hours 1/2/3/4th day 2 week blood 2 week urine
Samples	Blood, urine, sweat, milk (periodic?)	Blood and urine (esposure, and post during day, evening, & at wakeup)	Blood and urine (esposure, and post throughout 20 hours post)	Same but fractionated blood and urine, integrated urine	Same but fractionated blood and urine, integrated urine, expired breath
Samples/subject	undefined	2 control, 14-20 bloods all urine ad lib	2 control, 14-20 bloods all urine ad lib	10 controls, 21 bloods, 25-30 urines, 9 vials/BS 5 vials/US	10 controls, 25 bloods, 25-30 urines, 9 vials/BS 5 vials/US
Vials/subject (approximate)	25 (estimate)	30-40 (estimate)	50-60 (estimate)	350-375	350-375
Shipping destinations	1	1	1	3	3
Other	ECG and ICG % body fat	ECG and ICG % body fat	ECG and ICG % body fat Cognitive	ECG and ICG % body fat Cognitive	ECG and ICG % body fat Cognitive

Based on the approved protocols of May and September 1995, the study was executed as follows:

Subjects were instructed to refrain from drinking alcoholic beverages (wine, liquor, beer, coolers, etc.) for three days prior to and four days after each experiment session. On each day of the experiment, subjects ate a light, low-fat breakfast at about 7:00 am. Two subjects participated on each experiment day with a 10-minute delay between experiment procedures for the two subjects to help ensure on-time acquisition of each subject's blood samples.

Subjects arrived at the RTI Human Studies Facility (about 8:00 am) and changed into experiment attire. (They were asked to wear light, comfortable clothing and to bring a change of clothes to sleep in). All female subjects were given a serum pregnancy test; then asked to sign a statement assuring non-pregnancy. Subjects were instrumented with ECG electrodes and thoracic electrical impedance electrodes. Then, the consulting physician or medical technologist then inserted a catheter into a lower arm vein for obtaining repeated blood samples.

Subjects then entered the exposure chamber. Each subject sat at rest for a minimum of ten minutes to establish stable baseline physiological signals and to insure integrity of the venous catheter site. During this period, subjects participating in the EPA cognitive effects test were evaluated for visual acuity, visual contrast sensitivity, and color blindness, and were instructed on interaction with the cognitive test software.

At 10:00 am (or shortly after), subjects began receiving a blinded exposure of either 50 or 100 ppm TCE while sitting at rest. Each exposure lasted 4 hours. Following exposure, subjects remained in the chamber breathing clean air for 18 additional hours, for a total of 22 hours in the chamber. The electrocardiogram and impedance cardiogram were recorded throughout the 22-hour experiment (except during bathroom breaks).

Prior to, during, and after TCE exposure, blood samples were taken from the venous catheter or by venipuncture according to the schedule listed in Table 2. About 10 minutes before each blood sample, subjects performed the EPA cognitive test battery and symptom questionnaire. Immediately after each blood sample, subjects were asked to produce a corresponding urine sample.

Subjects were not permitted any food or drink during the 4-hour exposure segment. Following exposure, subjects were given lunch and dinner and were permitted juice, water, or non-caffeinated beverages. During the post-exposure period, subjects were permitted to view videos and read as desired between blood and urine sampling, and retired to bed about 10:00 PM. During the night, subjects were awakened every two hours for the scheduled blood and urine samples.

In the morning, subjects were awakened for their final sampling, given juice and a snack if desired, had their catheter withdrawn and catheter site inspected, were deinstrumented, and permitted to shower.

Before release, subjects were provided with urine collection containers and portable coolers, and instructed to collect all urine throughout each of the first 4 days post exposure, and to label each container with the date and time of collection. Subjects returned each of the following 3 mornings to RTI to return the self-collected urine samples and have a daily blood sample by venipuncture. Subjects also returned 2 weeks post-exposure for a final blood sample and random urine sample.

In a subset of the exposure studies, during and after the exposures, breath samples were also taken by exhaling via a tube into a partially-evacuated stainless-steel container. Breath sampling occurred shortly after each blood sample, and the containers were taken to EPA for gas analysis.

Table 2. Planned and actual blood sampling schedule for subject #208 (t(0)=10:25 am).

Sample Number	Sample ID	Date	Exper. Time hh:mm	Relative Time hh:mm	Collection Time hh:mm	Collection time from exposure start hh:mm	Comments
1	BS208-1	10-2-95	8:30	pre-exposure	8:30	pre-exposure	
			10:10				Air Mask on
		Start TCE	**10:25**	**0:00**		0:00	**Air Mask off**
2	BS208-2		**10:55**	**0:30**	10:55	0:30	
3	BS208-3		**11:25**	**1:00**	11:25	1:00	
4	BS208-4		**12:25**	**2:00**	12:25	2:00	
5	BS208-5		**13:25**	**3:00**	13:25	3:00	
6	BS208-6	**End TCE**	**14:25**	**4:00**	14:25	4:00	**Air Mask on**
7	BS208-7		14:40	4:15	14:40	4:15	to end
8	BS208-8		14:55	4:30	14:57	4:32	exposure
9	BS208-9		15:25	5:00	15:25	5:00	
10	BS208-10		16:25	6:00	16:25	6:00	
11	BS208-11		18:25	8:00	18:25	8:00	
12	BS208-12		20:25	10:00	20:25	10:00	
13	BS208-13		22:25	12:00	22:25	12:00	
14	BS208-14	10/3/95	0:25	14:00	0:25	14:00	
15	BS208-15		2:25	16:00	2:27	16:02	
16	BS208-16		4:25	18:00	4:25	18:00	
17	BS208-17		6:25	20:00	6:25	20:00	
18	BS208-18		8:25	22:00	8:25	22:00	
19	BS208-19	10/4/95	8:25	46:00	13:25	51:00	
20	BS208-20	10/5/95	8:25	70:00	16:30	78:05	
21	BS208-21	10/6/95	8:25	94:00	15:15	100:50	
22	BS208-22	10/13/95	day 11		15:55		

2.3 Trichloroethylene Exposure

2.3.1 Chamber TCE Concentration Maintenance Procedures

Approximately 10 minutes prior to the intended exposure start, subjects were placed on compressed breathing air using a flow-through face mask. The flow rate was adjusted to provide an excess of flow over peak inspiratory flow rate. A precision positive-displacement metering pump (Pro-Spense™) was turned on, and pure TCE (Fisher Scientific, Stabilized, Certified ACS) was pumped from a 4-liter jug into an insulated 0.25" diameter, 6' long, stainless-steel tube that was wrapped with heating tape. The tubing was maintained at 200°F by a Fuji Electric temperature controller and a type-J thermocouple cemented to the tubing approximately one foot from its distal end. The tubing temperature was chosen to be above the boiling point of TCE (87°C), but low enough to avoid surges of liquid within the tubing. A second monitoring thermocouple was attached to the evaporator tube approximately at its center to ensure that the liquid-containing portion of the tube did not overheat. The tubing was attached to a multiport (5) sparger that resided in the supply-air duct for the chamber. Both the sparger tubing and the dispersion nozzles were maintained at 240°F by separate Fuji Electric controllers and thermocouples placed appropriately. The sparger tubing and nozzles were operated at a higher temperature to ensure that there would be no points of condensation within the system and thus, that the mass-flow-rate perturbations of TCE would be small.

Airflow in the chamber during the exposure was held constant by the environmental-control system. Concentration of TCE was controlled open-loop (actually, man-in-the-loop) by making manual adjustments to the TCE flow rate and monitoring the results on both the Miran readout and the dosing-system logging display. Atmospheric pressure was observed to be relatively stable (less than 2 mmHg variation) on all of the experiment days during the exposure periods. The chamber air was well-mixed by a pair of circulating fans placed in the chamber.

At the end of exposure for both subjects, the TCE pump was reversed and set to maximum speed to remove liquid from the evaporator tubing, the power to the evaporator and sparger was removed, the chamber supply-air flow was increased from nominally 400 cfm to approximately 1200 cfm, and the chamber circulating fans were turned off to speed washout. In later experiments, the chamber-flow step was omitted, because we found that the sudden change in air flow upset the humidity control. (The chamber is optimized for an air flow of approximately 800 cfm and has the capacity to maintain 90% relative humidity over a 40°F range. This capacity, in combination with the relatively sluggish dynamics of the humidity-control system, results in humidity oscillations when temperature, humidity, or air flow setpoints are changed too rapidly.)

Miran-based chamber concentration of TCE was logged continuously before, during, and after exposure. The logging records contain measurements of chamber temperature, relative humidity, air flow, atmospheric pressure, and TCE concentration at approximately one-minute intervals. Chamber partial pressure of TCE is calculated and recorded for each measurement, based on the two calibration factors for the Miran (at 50 and 100 ppm). Fractional concentration is also calculated from atmospheric pressure and reported with a multiplier of 10,000 (to accommodate range-display limitations in the logging-system display).

An anomaly in the Miran behavior was noted and reported in correspondence with WPAFB personnel. At the end of exposure (or within half-an-hour or so), we routinely turned off the sampling pump for the Miran. The chamber had washed out, and there was no further introduction of TCE. During preparation of the logging files, we discovered that the output signal from the Miran began to rise after the pump was turned off, reaching the equivalent of 3-4 ppm by the time the logging system was turned off at the end of the experiment (approx. 24 hours after logging began). Turning on the pump again

caused the indicated concentration to rapidly return to <1 ppm, suggesting that the Miran's measurement chamber had adsorbed TCE and was releasing it after the sample pump was turned off. We therefore initiated post-exposure spot-sampling for two experiments to verify that the chamber concentration was not increasing. The GC/MS verified that we did indeed achieve a clean (<1 ppm) washout of the chamber, and that the anomalous readings arose from the Miran. We therefore changed our procedure to allow the Miran sampling pump to continue running throughout the experiment. The post-exposure concentrations logged remained in the 1 ppm range, a value that is near the limit of resolution of the system. For purposes of modeling the data from all experiments, the post-exposure chamber concentration may be taken to be 0 ppm once the logged TCE concentration dips to 1 ppm.

2.3.2 Collection of Chamber Air Samples for TCE Measurement by GC/MS

Chamber air samples were collected on Tenax cartridges and analyzed by GC/MS during each TCE exposure experiment as an independent measurement to confirm the dosing levels as indicated by the Miran IR spectrometer. The sample collection and analysis methods are described below.

A Dupont P-4000 air pump was connected to a 1/4 inch stainless steel "Tee" fitting that was attached to the sampling bulkhead of the chamber. A septum was inserted into the open port of the "Tee" to provide a sealed opening from which to withdraw an air sample using a syringe. The P-4000 pump was turned on and chamber air was pulled through this sampling manifold at a flow rate of approximately 3.5 liter/min. Prior to collecting an air sample, the manifold was purged for approximately 5-10 minutes with chamber air to allow equilibration of the manifold components. Air from the chamber was drawn through the manifold from a length of 1/4 inch Teflon tubing that terminated inside the chamber adjacent to the Miran sampling tube.

A Pressure-lok gas-tight 2.00 ml glass syringe was used to transfer the chamber-air sample to a Tenax-sorbent sampling cartridge. The syringe was equipped with a valve that sealed the syringe between filling and injection onto the Tenax cartridge. A 1.00 ml aliquot of chamber air was injected onto the cartridge if the chamber was being dosed at 100 ppm and a 2.00 ml aliquot was injected onto the cartridge if the chamber was being dosed at 50 ppm. This provided equivalent masses on the cartridges for the two dosing levels. These small volumes were necessary to ensure a reasonable mass of TCE for analysis without the need for a complex dilution system. Approximate TCE mass on the cartridges was 500 to 600 ng. The syringe was purged multiple times with chamber air to condition it prior to injection onto the sorbent bed. For injection onto the Tenax cartridge, a 2 inch needle was installed on the syringe and the air slowly injected into the Tenax bed. Triplicate samples were collected and analyzed from each experiment.

Samples were analyzed by thermal desorption, gas chromatography/mass spectrometry (GC/MS). Quantitation was based on response factors determined from replicate analyses of a single calibration point. Three calibration cartridges prepared at 510 ng/cartridge were analyzed prior to each set of samples to determine the relative response factor for TCE. Standards were prepared by a flash evaporation method on Tenax cartridges using a TCE in methanol standard solution. A 1.0 ml aliquot of the standard solution was injected into a heated flash evaporation system with a helium gas flow of 60 ml/min. The vaporized solution and helium gas were passed through a Tenax cartridge to trap the TCE. The mass of TCE loaded onto the standard cartridges was equivalent to the mass of TCE in a 1.00 ml volume of 100 ppm or 2.00 ml volume of 50 ppm gas.

In addition to the samples and calibration cartridges, Tenax cartridge blanks and certified 50 ppm and 100 ppm gas calibration mixtures (Scott Specialty Gases) were analyzed. The results of the analyzed cartridges appear in the appendix.

Certified gases were not available in time to calibrate the Miran for the first two exposure experiments, so the nominal calibration supplied by WPAFB with the instrument were used. These curves were inadequate to use as calibration information, because no atmospheric pressure information was supplied with the data. Because the Miran responds to partial pressure rather than to fractional concentration (ppm), we chose to control the partial pressure of TCE in the chamber, rather than the fractional concentration. This is appropriate for modeling also, because the driving force in the lungs is the differential partial pressure of TCE between the alveolar gas and the blood, not the fractional difference.

The certified gas mixtures were used to establish the Miran output voltage for each of the target concentrations (50 and 100 ppm). A zero was obtained by flushing the Miran with bottled breathing air at a rate of approximately 1 liter/min. The zero-offset control was adjusted to null the instrument output after the signal appeared stable on a 3-1/2-digit voltmeter (i.e., no discernible monotonic drift in readings). Similarly, the Miran was flushed with each of the certified gases and the respective output signals were recorded. The relation between TCE concentration and output voltage is not linear, but because only two chamber concentrations were to be used, the two-point calibration (zero, and reference value for each concentration of interest) is an adequate method.

TCE partial pressure (mmHg) was calculated by multiplying the certified fractional concentration of TCE by the atmospheric pressure at the time of calibration. The Miran output voltages at the nominal 50 and 100 ppm concentrations, divided into the partial pressures gave calibration sensitivities for the chamber environmental logging system. A digital voltmeter was attached to the Miran at all times to provide continuous readout (the sensitivities were fortuitously approximately 1 mV/ppm, so a direct estimation was visible at all times). Spot checks of the Miran's zero and 100 ppm sensitivity showed no discernible drift throughout the experiment series (the instrument was allowed to remain on for the entire period July-December, with only the sampling pump being turned off).

The resolution of the A/D conversion in the data-logging computer is 12 bits, and the voltage input range of the converter is 0-5 volts, giving a resolution of $5/2^{12}$ or 1.221 mV/bit. The equivalent resolutions for the measurements, shown in Table 3, are the products of the sensitivity at the calibration point and the voltage-conversion resolution. Data in the logging record that imply a resolution of greater than one part in 5000 (actually, 4096) are simply the result of the calculation algorithms being unaware of the base resolution of the raw data.

Table 3. The equivalent resolutions for TCE and environmental measurements.

Measurement	Range	Units	Nom. Sensitivity	Meas. @	Resolution/bit
Miran, @ nom. 100 ppm	0 - 760	10^{-4} mmHg	701.9×10^{-4} mmHg/V	74.1×10^{-3} mmHg	85.7×10^{-6} mmHg
Miran, @ nom. 50 ppm	0 - 760	10^{-4} mmHg	752.9×10^{-4} mmHg/V	37.2×10^{-3} mmHg	91.9×10^{-6} mmHg
Miran, @ PB = 751.4 mmHg	0 - 100	ppm	100.2 ppm/V	98.6 ppm	0.122 ppm
Miran, @ PB = 751.6 mmHg	0 - 100	ppm	93.4 ppm/V	49.5 ppm	0.113 ppm
Barometric Pressure	0 - 1000	mmHg	200 mmHg/V	760 mmHg	0.244 mmHg
Temperature	0 - 50	°C	10°C/V	25°C	0.012 °C
Relative Humidity	0 - 100	%	20%/V	Not calibrated	0.024%/V

2.4 Blood and Urine Sample Collection

Blood and urine samples were acquired as described in the protocol (see Section 2.2) at or as close to the designated sampling times as possible (see Table 2). Because of the long period between blood samples without a venous drip (i.e., saline infusion), many of the catheters developed blood clots. To remedy this situation, either a new catheter was inserted at another site or subsequent blood samples were taken be venipuncture. Either remedy occasionally resulted in delayed or missing samples.

Preparation of blood and urine samples for off-site TCE and TCE-metabolite analysis required that samples be split into sub-samples, treated with a reagent, and flash-frozen as soon as possible after each sample was taken. Sub-sample vials were treated with the appropriate reagent within one week before each experiment, labeled, and set aside.

The general procedure for processing each blood and urine sample was as follows:

1. Collect blood in a 4 ml heparinized tube (green top) and record the time of the draw on the sample collection sheet. Pipette 5 subsamples immediately into vials according to Tables 4 and 6. (Labeling scheme: BSsbj#-sample#-vial#). Freeze immediately in liquid nitrogen.

2. Spin down the remaining blood sample and pipette the remaining three blood subsample: 0.1 ml plasma, remaining plasma, and 0.3 ml red blood cells (RBCs).

3. Collect a complete voided urine sample and measure the total volume. Record the time of the sample collection and total volume on the sample collection sheet. (Labeling scheme: USsbj#-sample#-vial#). Pipette 4 subsamples into vials according to Tables 5 and 7 and freeze in liquid nitrogen. Pour a 10-15 ml sample into a plastic vial (#5), and place it in a standard freezer

4. Place all samples in 4 ml amber Teflon-coated septa screw cap vials and freeze immediately in liquid nitrogen. Place serum in 12x75 plastic shipping tubes and place it in a standard freezer.

5. Transfer samples from the liquid nitrogen to dry ice for sorting.

6. Place the samples, in sample order, in cardboard boxes and hold in dry ice for transportation to the -80°C freezer in the chemistry building.

7. Ship samples in dry ice to their appropriate destinations in accordance with the IATA regulations.

Shipping addresses were follows:

WPAFB: Dr. Richat Abbas (Tel: 513-255-2704)
Wright Patterson Air Force Base, OL AL HSC/OET Building 79
2856 G Street, Wright-Patterson AFB, Ohio 45433-7400

* Sample to be shipped to: Dr. Larry Lash (Tel: 313-577-0475)
Dept. of Pharmacology, Wayne State University, School of Medicine
540 E. Canfield Ave., Detroit, Michigan 48210

** Sample to be shipped to: Dr. Debasis Bagchi (Tel: 402-280-1206)
Dept. of Pharmaceutical & Administrative Science, School of Pharmacy & Allied Health
2500 California Plaza, Omaha, Nebraska 68178

Table 4. Blood sample vial preparation and sample collection.

Vial Number	Sample Name	Preservative	Solvent	Sample Volume
1	TCE (WPAFB)	0.2 ml 20% lead acetate	0.1 ml H_2O	**0.2 ml blood**
2	CH & $TCOH_{free}$ (WPAFB)	0.2 ml 20% lead acetate		**0.2 ml blood**
3	$TCOH_{total}$ (WPAFB)	0.5 ml conc. sulfuric acid		**0.2 ml blood**
4	TCA & DCA (WPAFB)	0.1 ml 20% lead acetate		**0.1 ml blood**
5	Glutathione (LL)*	0.1 ml 70% perchloric acid		**0.5 ml blood** use µfuge vials
6	Extra blood (WPAFB)	0.5 ml 20% lead acetate		**0.5 ml blood**
7	RBC's (WPAFB)	0.3 ml 20% lead acetate		**0.3 ml RBC's**
8	Extra plasma (WPAFB)			**extra plasma**
9	Oxidative Stress (DB)**			**0.1 ml plasma**

Labeling scheme: BSsbj#-sample#-vial#.

An extra green top tube should be drawn at 12, 22, 46, 72, and 94 hours, then frozen in the freezer as whole blood. This sample is to be sent to WPAFB so it can be forwarded to Germany.

* Sample to be shipped to: Dr. Larry Lash (Tel: 313-577-0475)

** Sample to be shipped to: Dr. Debasis Bagchi (Tel: 402-280-1206)

Table 5. Urine sample vial preparation and sample collection.

Vial Number	Sample Name	Preservative	Urine Volume
1	TCA & DCA (WPAFB)		**2 ml urine**
2	Glutathione (LL)*	0.4 ml 70% perchloric acid	**2 ml urine**
3	Oxidative Stress (DB)**		**2 ml urine**
4	$TCOH_{total}$ (WPAFB)		**2 ml urine**
5	$TCOH_{free}$ (WPAFB)		**15 ml urine in plastic vial**

Labeling scheme: USsbj#-sample#-vial#.

* Sample to be shipped to: Dr. Larry Lash (Tel: 313-577-0475)

** Sample to be shipped to: Dr. Debasis Bagchi (Tel: 402-280-1206)

Table 6. Control blood, plasma, and serum sample vial preparation and collection.

Vial Number	Sample Name	Time of Draw	Sample Volume
1	Plasma DCA, MCA (WPAFB)	Before exposure	**2 ml plasma** 2 vials
2	Plasma Oxidative Stress (DB)**	Before exposure	**0.2 ml plasma**
3	Serum Clin. Chemistry (WPAFB)	Before exposure, end of 1st day, and end of 4th day.	**1 ml serum** (in 12x75 shipping vial)
4	Blood (WPAFB)	Before exposure	**2 ml blood** 4 vials
5	Glutathione (LL)*	Before exposure	**0.5 ml blood** use µfuge vials
6	RBC's (WPAFB)	Before exposure	**2 ml RBC's separated plasma** 2 vials

Labeling scheme: BCsbj#-sample#-vial#.

* Sample to be shipped to: Dr. Larry Lash (Tel: 313-577-0475)

** Sample to be shipped to: Dr. Debasis Bagchi (Tel: 402-280-1206)

Table 7. Urine control sample vial preparation and sample collection.

Vial Number	Sample Name	Time of Collection	Urine Volume
1	Urine (WPAFB)	Before exposure	**2 ml urine** 3 vials
2	Oxidative Stress (DB)**	Before exposure	**2 ml urine**
3	Urine Clin. Chemistry (WPAFB)	Before exposure, end of 1st day, and end of 4th day.	**2 ml urine**
4	Glutathione (LL)*	Before exposure	**2 ml urine**

Labeling scheme: UCsbj#-sample#-vial#.

* Sample to be shipped to: Dr. Larry Lash (Tel: 313-577-0475)

** Sample to be shipped to: Dr. Debasis Bagchi (Tel: 402-280-1206)

2.5 Cardiac Function Measurements

For noninvasive cardiac function measurements, ECG leads V_5, the impedance cardiogram (ΔZ, ICG), the ICG first derivative (dZ/dt) and the mean thoracic electrical impedance (Zo) were monitored continuously throughout each experiment, sampled at 400 Hz per channel, and stored on computer disk. The acquired cardiac signals were analyzed off-line using RTI's WVSHELL software according to methods previously described (Kizakevich et al., 1989; Kizakevich et al., 1993a). At three-minute intervals throughout the acquired datasets, a 30-second ensemble average of the ECG, ΔZ, dZ/dt, and Z_0 signals was initiated. During ensemble averaging, successive cardiac cycles were extracted, displayed, and automatically accepted or rejected according to R-R interval to enhance signal quality. After thirty-two (32) qualified cycles were averaged, the six ensemble-averaged waveforms were automatically analyzed for specific waveform features, i.e. systolic time intervals (Sheps, 1982), peak-systolic dZ/dt amplitude and mean-systolic ICG acceleration (Figure 2). The primary ECG and ICG variables were:

RATE	heart rate (beats/min)
dZ/dt_{peak}	peak systolic amplitude of dZ/dt (Ω/s)
TZavo	time from ECG Q-wave to aortic valve opening [dZ/dt_{up}] (ms)
TZpeak	time from ECG Q wave to dZ/dt_{peak} (ms)
TZx	time from ECG Q-wave to aortic valve closing [dZ/dt_X] (ms)
ACCEL	ICG acceleration (cardiac contractility) (Ω/s^2)

Stroke volume and cardiac output were calculated using an empirical model relating TEI measurements to changes in thoracic blood flow (Kubicek et al., 1966; Sherwood et al., 1993; Everson et al, 1991):

$$SV = \rho \cdot (L/Z_0)^2 \cdot dZ/dt_{peak} \cdot (TZx - TZavo) \quad \text{cc}$$

$$CO = RATE \cdot (SV / 1000) \quad \text{L/min}$$

where the thoracic electrical impedance electrode separation, L, was measured for each subject and the blood resistivity, ρ, was assumed to be 135 Ω-cm (Kubicek et al., 1966). Measured and calculated variables (a data record) were stored to disk, while selected variables were appended to time-series trend waveforms. A composite of the analyzed ensemble-averaged signals and trend variables was then displayed and a printed copy made for the experiment archive.

Several processes were performed to assure data quality and prepare a more tractable data set for statistical analysis. Qualified data sets were then converted to an Excel spreadsheet format for archival storage and further analysis. The graphic trend of each key variable was reviewed to identify trend outliers, measurement errors, missing data records, experiment procedure problems, and other easily observable inconsistencies in the raw acquired data. Missing data were repaired by substituting estimated data by assuming a linearity between pre- and post-missing data records. If the missing data series exceeded two samples, the data were left as missing. Time-series data for each variable were smoothed using a 3-point weighted filter.

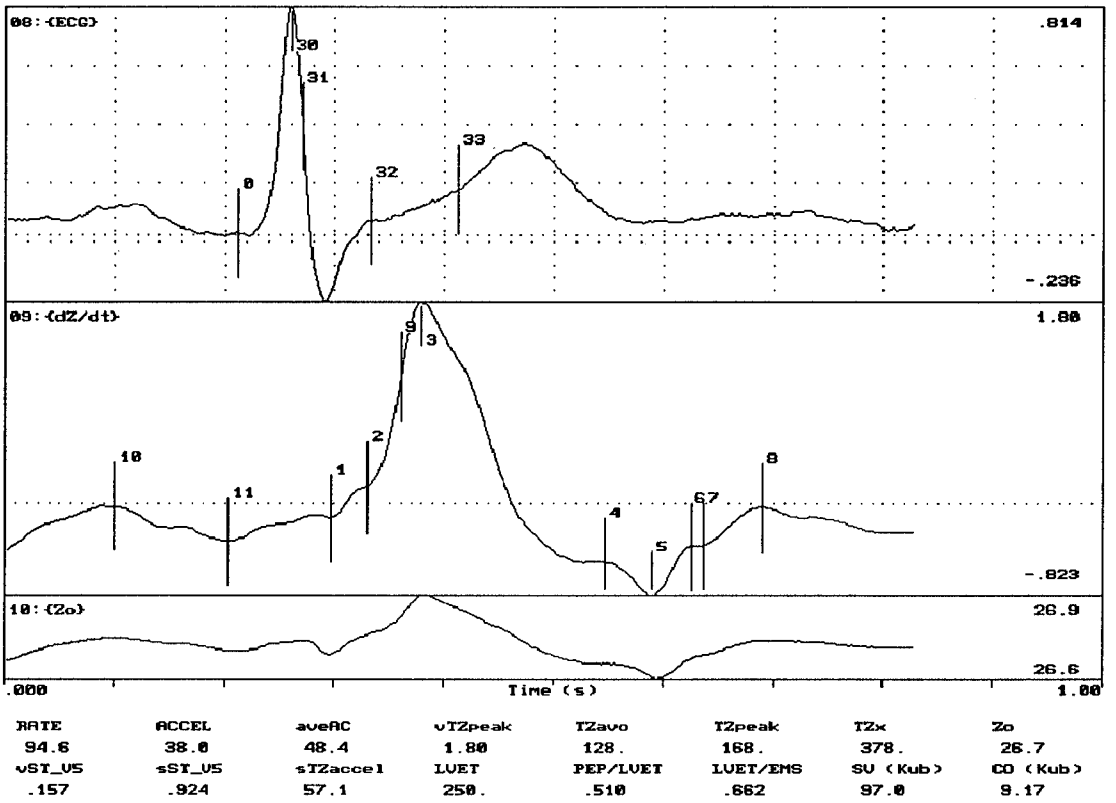

Figure 2. Analysis of cardiac function data. The physiological waveforms are the ECG, impedance cardiogram first derivative (dZ/dt), and mean thoracic electrical impedance (Zo) for measurement of cardiac timing and amplitude variables. Primary waveform features are onset of systole (#0), aortic valve opening (#2), peak ejection (#3), and aortic valve closure (#5).

2.6 Cognitive Performance and Symptom Measurements

A secondary objective was to extend the study with a variety of cognitive tests and short-term health effects using a computerized testing system provided by the EPA. These tests were designed to determine any short-term effects on mental awareness and neurological performance. The tests included, for example, simple mathematics, memory recall, and hand-eye coordination tasks, and on-line symptom questionnaires. The cognitive and short-term health effects data were taken, analyzed, and retained by EPA scientists.

2.7 Results

2.7.1 Subjects

Subject recruitment and qualification was done by placing an advertisement in a local Chapel Hill newspaper. A separate telephone line with voice mail was installed to receive phone messages. The messages were returned in the order in which they were received. Subjects were prequalified over the phone by informing them of the study protocol and by briefly reviewing their medical history. The telephone interactions were as follows:

Total number of different phone inquiries: 247

Total number of females: 144
Total number of males: 103

Phone calls actually answered: 72
Subjects qualified: 33
Subjects not qualified or not interested: 39

Subjects phone qualified and medical physicals performed: 21

Twenty-one (21) subjects were telephone prequalified and entered into the study. Each gave informed consent and completed a medical history and physical examination before participating in any experiment procedures. Of the 21 subjects, two were excluded based on their medical history or physical examination, and two could not be scheduled for the experiments. Therefore, seventeen (17) subjects participated in the TCE exposures. A summary of participating subjects is as follows

Subjects disqualified after physical: 2

Subjects enrolled in 50 ppm, 100 ppm, or both exposures: 17

Subjects completing the entire experiment: 13
Subjects completing the exposure but did not return for the 12 day post exposure sample: 4

Subjects exposed at both exposure levels (50 ppm and 100 ppm): 4

Total subjects exposed at 50 ppm: 5
Total subjects exposed at 100 ppm: 16

Anthropometric characteristics and the exposure schedule for subjects participating in the experiment are presented in Tables 8-10. The primary subject-dependent recruitment criteria was to include equal numbers of men and women ranging from 18 to 35 years of age. The final study population included 8 females and 9 males. The age range was 20 to 31 years of age for females and 20 to 36 years of age for males. The 36 year old male was found out after his participation was nearly completed, so he remained in the study. Females, on average, had a higher percent body fat ranging from 21 to 35% (mean=27%) as compared to the 6 to 27% range (mean=15%) in males.

Table 8. Distribution of anthropometric and exposure schedule for study population.

Date	Subject #	ppm	% Body Fat	Height	Weight	Heart Rate	Resistance	Reactance	Race	Sex	Age	Activity level	Start Time
7/17/95	101	60	32	64	146.5	72	587	75	w	f	30	H	11:15
	201	60	14	73	156.5	60	519	70	b	m	23	H	11:20
7/24/95	102	50	24	68	137	80	593	65	w	f	24	M	10:50
	202	50	6	66	115	72	521	80	w	m	22	L	10:55
7/31/95	103	100	17	68.5	157	56	486	76	w	m	23	H	10:00
	203	100	14	72	181	48	428	82	w	m	23	H	10:05
8/7/95	104	100	21	65	126.5	68	565	66	w	f	31	H	10:35
	204	100	14	74.5	182	56	453	63	b	m	36	H	10:40
8/14/95	105	100	32	64	146.5	72	587	75	w	f	30	H	10:10
	205	100	14	73	156.5	60	519	70	b	m	23	H	10:15
8/21/95	106	50	27	66	152.5	66	580	90	w	m	20	L	10:05
	206	no subject											
9/25/95	107	100	23	66	122	84	638	72	w	f	20	L	10:05
	207	100	33	66.5	136	80	717	81	w	f	22	M	10:10
10/2/95	108	100	35	65	148	72	638	98	w	f	29	H	10:20
	208	100	18	75	161	66	570	98	w	m	33	L	10:25
10/9/95	109	100	24	68	137	80	593	65	w	f	24	M	10:15
	209	100	6	66	115	72	521	80	w	m	22	L	10:20
11/6/95	110	100	26	65	139	66	581	83	w	f	28	L	11:30
	210	100	10	69.5	134	64	517	67	w	m	23	L	11:35
11/27/95	111	100	23	61	107	72	652	85	w	f	29	L	10:00
	211	100	18	71	156	56	531	81	w	m	25	M	10:05

Activity Level Codes:

L=Light: no organized physical activity during leisure time with three to four hours of walking or standing per day.

M=Moderate: sporadically involved in recreational activities such as weekend golf or tennis, occasional jogging, swimming or cycling.

H=Heavy: consistent job activities of lifting or stair climbing or participating regularly in recreational/fitness activities such as jogging, swimming, or cycling at least 3 times per week.

Table 9. Female Study Population Characteristics

Date	Subject #	% body fat	Height	Weight	Heart rate	Race	Sex	DOB	Age	act. level
7/17/95	101	32	64	146.5	72	w	f	8/24/65	30	H
7/24/95	102	24	68	137	80	w	f	1/18/71	24	M
8/7/95	104	21	65	126.5	68	w	f	7/11/64	31	H
9/25/95	107	23	66	122	84	w	f	11/13/74	20	L
	207	33	66.5	136	80	w	f	11/29/72	22	M
10/2/95	108	35	65	148	72	w	f	8/9/66	29	H
11/6/95	110	26	65	139	66	w	f	2/21/67	28	L
11/27/95	111	23	61	107	72	w	f	3/10/66	29	L
Max		**35**	**68**	**148**	**84**				**31**	
Mean		**27**	**65**	**133**	**74**				**27**	
Min		**21**	**61**	**107**	**66**				**20**	

Table 10. Male Study Population Characteristics

Date	Subject #	% body fat	Height	Weight	Heart rate	Race	Sex	DOB	Age	act. level
	201	14	73	156.5	60	b	m	6/30/72	23	H
	202	6	66	115	72	w	m	4/14/73	22	L
7/31/95	103	17	68.5	157	56	w	m	6/4/72	23	H
	203	14	72	181	48	w	m	7/5/72	23	H
	204	14	74.5	182	56	b	m	12/14/58	36	H
8/21/95	106	27	66	152.5	66	w	m	9/8/74	20	L
	208	18	75	161	66	w	m	5/31/62	33	L
	210	10	69.5	134	64	w	m	1/13/72	23	L
	211	18	71	156	56	w	m	11/10/70	25	M
Max		**27**	**75**	**182**	**72**				**36**	
Mean		**15**	**71**	**155**	**60**				**25**	
Min		**6**	**66**	**115**	**48**				**20**	

Activity Level Codes:

L=Light: no organized physical activity during leisure time with three to four hours of walking or standing per day.

M=Moderate: sporadically involved in recreational activities such as weekend golf or tennis, occasional jogging, swimming or cycling.

H=Heavy: consistent job activities of lifting or stair climbing or participating regularly in recreational/fitness activities such as jogging, swimming, or cycling at least 3 times per week.

2.7.2 Trichloroethylene Exposure

Target concentrations for the 4-hour TCE exposures were 50 ppm on three experiment days and 100 ppm. on eight experiment days. Actual TCE chamber levels were calculated from continuous recording of the Miran and environmental control analog signals. Two sets of the exposure data were lost, the first due to an equipment failure and the second due to operator error.

Table 11 presents a summary of TCE exposure conditions including mean exposure, the standard deviation of the mean exposure, the coefficient of variation of the mean exposure (CV), the mean offset from the target concentration (50 or 100 ppm) and the percent offset from the target concentration.

On average, the TCE exposure concentrations were within 4% of target experimental conditions. Since the PB-PK model analysis will use the actual time-series TCE concentration data, controlling the exposure to within 5% of nominal target levels was more than adequate. This was especially true for a single-pass air handling system with a fairly chaotic minute-by-minute air flow pattern and a semi-automated TCE exposure control system.

2.7.3 Trichloroethylene Instrument Calibration

Calibration data were supplied with the GFE Miran IR spectrometer, at wavelength and sensitivity settings that are appropriate for the TCE concentrations used in the study. Because the instrument had been shipped, and because no atmospheric-pressure data accompanied the calibration information, we obtained reference gases for local calibration purposes.

As a quality-control measure, we also had RTI's analytical chemistry group analyze samples of the chamber air during exposure and later, after chamber washout. These samples were collected on Tenax cartridges and analyzed by gas chromatography and mass spectrometry. The reference samples for calibrating the GC/MS were prepared for each sample run from liquid TCE.

In Figure 3, the Miran measurements of the TCE fractional concentrations are plotted against the GC/MS values. Because the spot-sample times represent a time period, rather than an instant, the Miran values used for comparison are the averages of the three sample-time values nearest the reported GC/MS sample times (ideally, the GC/MS sample time, one-minute before and one minute after). [The data points on the X-axis are the GC/MS data for which we had no corresponding Miran data - experiments 8 & 9, from which the logging-system data were lost] There is clearly considerable scatter in the GC/MS data relative to the Miran. We anticipated there might be an offset between the methods, but were surprised by the scatter, especially after the relatively good agreement between the methods for the certified reference gases (well within the stated analysis tolerance of the certified gases). The GC/MS sample preparation and the reference preparation each require a large number of manipulations and therefore introduce many opportunities for error.

The small sample volumes used to collect the chamber samples are suspected sources of error. The scatter of the 100 ppm data is higher than the scatter for the 50 ppm data, as might be expected if the total amount of TCE in the samples was not captured by the Tenax cartridge. Because the 50 ppm sample volume is twice that of the 100 ppm sample, a given loss of sample capture would result in a larger error for the smaller-volume sample, and this is consistent with the observed data.

In retrospect, we should have used the same certified gas mixture for each GC/MS calibration, rather than create a new liquid-based reference. This would have ensured that the reference was stable and would have constrained the sources of variability of the GC/MS method to the sample handling.

Table 11. Statistical summary of TCE exposure concentrations (for 9 of 11 experiment days)

Experiment #	Mean ppm	Std. Dev.	CV, %	Offset, ppm	Offset, %
1	55.12	1.20	2.17	5.12	10.2
2	52.97	1.78	3.35	2.97	5.9
3	105.44	1.74	1.65	5.44	5.4
4	102.54	2.00	1.95	2.54	2.5
5	101.41	1.54	1.52	1.41	1.4
6	49.27	1.29	2.62	-0.73	1.5
9	97.68	1.88	1.93	-2.32	2.3
10	100.96	2.62	2.60	0.96	1.0
11	103.24	3.08	2.98	3.24	3.2
MEAN		**1.90**	**2.31**	**2.07**	**3.71**

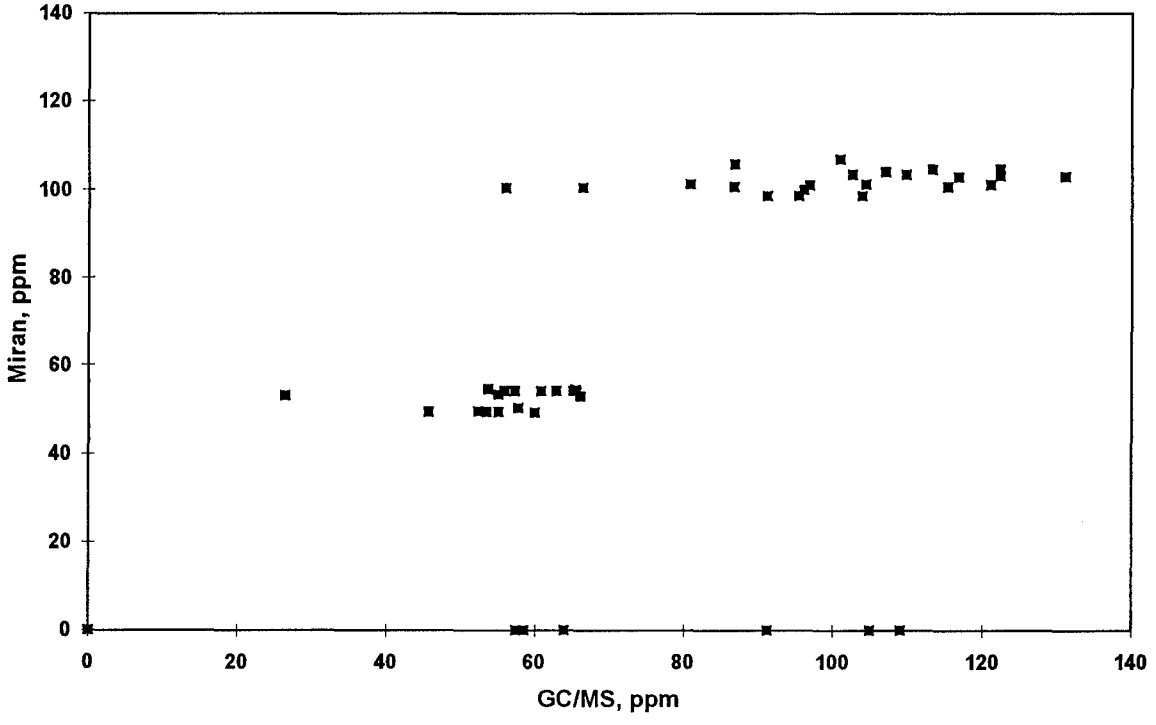

Figure 3. Miran versus GC/MS measurements of the TCE fractional concentrations.

2.7.4 Blood and Urine Sample Analysis

Analysis of blood and urine samples for TCE and TCE-metabolite concentrations was to be performed at WPAFB and WPAFB-designated laboratories. Furthermore, pharmacokinetic modeling and statistical analyses based of the experiment data were to be conducted at WPAFB. The final results of these analysis are not yet available to RTI, and are expected to be reported later.

2.7.5 Cardiac Function Data Analysis

Noninvasive cardiac function data were processed off-line using the methods described above. The resultant data set was extensive. Approximately 440 records were analyzed for each subject experiment day. Data trends plots were generated for each subject experiment. A multiple-subject integrated data set was prepared for shipment to WPAFB.

Data trends plots for the primary cardiac variables (heart rate, cardiac acceleration, stroke volume, cardiac output) are presented for Subject #206 (Figures 4-6). The missing data (spikes) occur shortly after each blood sample during periods when the subject was disconnected from the cardiac monitoring instrumentation to volunteer a urine specimen. Since the continuous cardiac data was analyzed on three-minute intervals, episodes whenever the subject was reconnected between sequential analysis times resulted in no missing data. Thus spikes do not occur for each corresponding blood sample.

3.0 CONCLUSIONS

3.1

This study was not intended to be a complete research project in itself, rather a collaboration between RTI and WPAFB scientists for physiologically-based pharmacokinetic modeling of TCE and TCE metabolism. As stated previously, the primary objective was to acquire biological samples and relevant experiment data under conditions of controlled TCE inhalation exposure, and to provide such samples and exposure data for analysis and physiologically-based pharmacokinetic modeling at WPAFB-designated laboratories. Furthermore, analysis of cognitive performance and subject symptom data have yet to be completed by EPA. Therefore, the implications of the results will not be fully known until the WPAFB TCE modeling and EPA analysis efforts are complete.

Considering these issues, several minor conclusions can be drawn from the experience of this work:

- The RTI Human Studies Facility exposure chamber can be used for TCE inhalation studies under semi-automated control with an average concentration precision of 3% and accuracy of 4%.

- Automated thoracic electrical impedance cardiography measurements can be used for 24-hour estimates of cardiac function at rest, during light activity, and during sleep.

3.2

Suggestions for future related work are:

- Recall the study subjects for an air-exposure control study to complete the EPA collaboration. EPA needs baseline data to better determine whether short-term 100 ppm TCE exposure adversely affects health, comfort, or cognitive performance. An air-exposure study was not part of the experiment plan since it was unnecessary for the collection of samples for PB-PK modeling.

- Replicate the experiment protocol with the subjects performing exercise during and/or after TCE inhalation. As illustrated in Figure 1, both cardiac output and respiratory ventilation are major factors in the TCE PB-PK model. Controlled exercise would provide data sets for PB-PK model validation under period of increased activity.

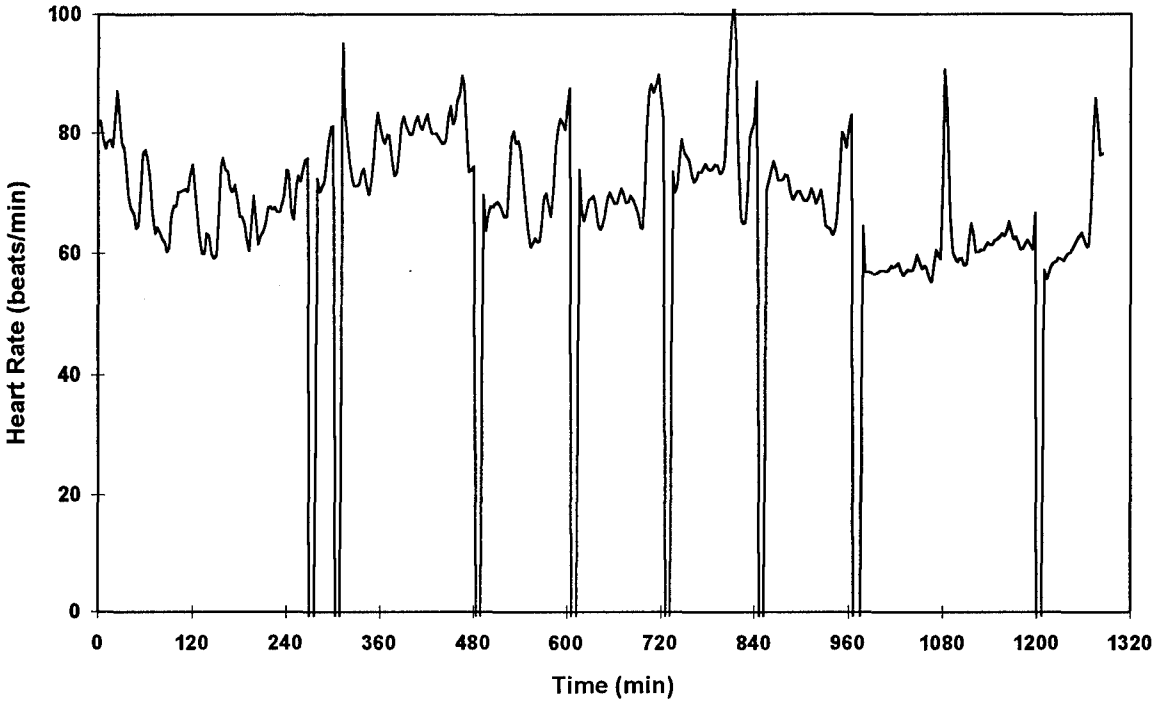

Figure 4. Example of Heart Rate time-series data (22-hours, Subject #206).

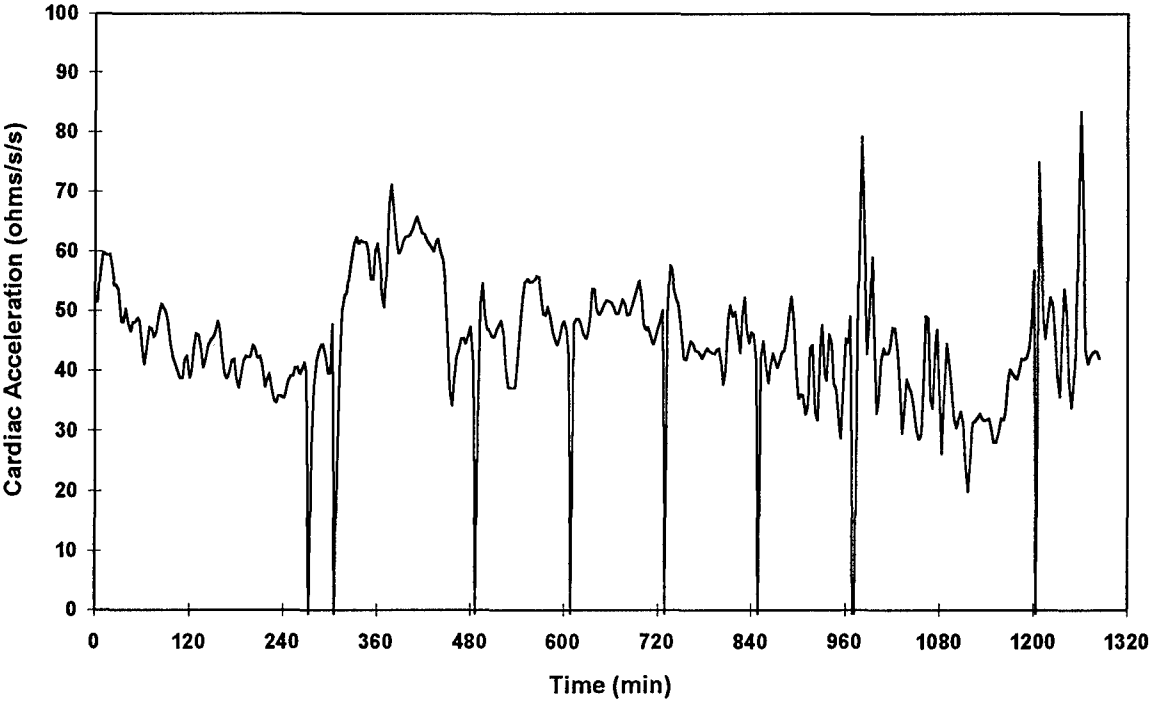

Figure 5. Example of Cardiac Acceleration time-series data (22-hours, Subject #206).

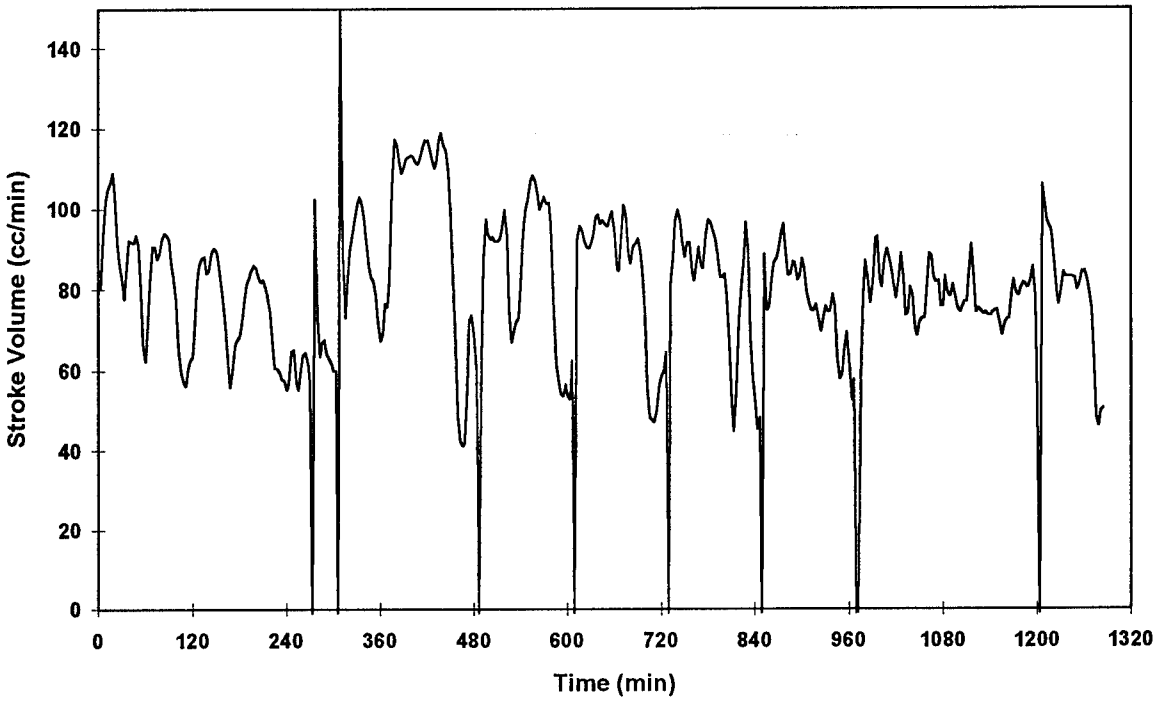

Figure 6. Example of Stroke Volume time-series data (22-hours, Subject #206).

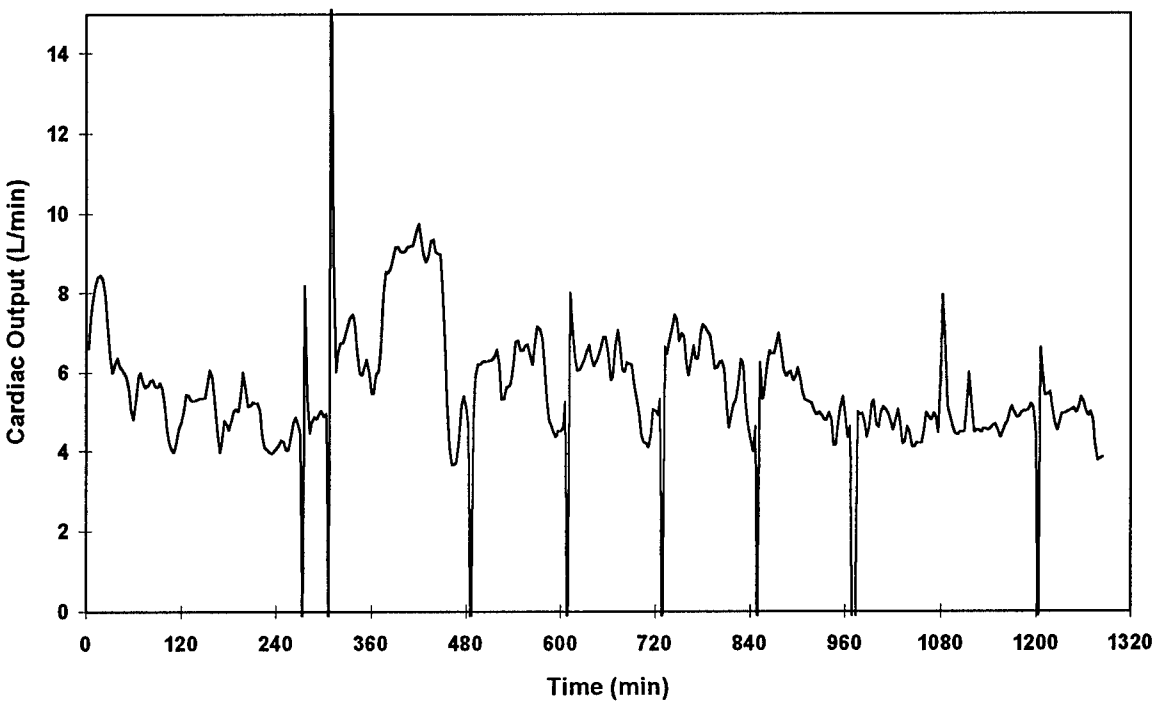

Figure 7. Example of Cardiac Output time-series data (22-hours, Subject #206).

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5.0 APPENDIX

Table 12. Chamber TCE concentrations based On Tenax cartridge analysis.

Sample Code	Date Collected	Time Collected	MIRAN Reading (volts)	Volume Collected (mL)	Measured Amount (ng/cart)	Calc. Chamber Conc. (ppm)	Average Conc. (ppm)	%RSD
7/14-50-1	7/14/95			2	726	67.06		
CART. BLANK	7/17/95			2	1.3	0.12		
EXPOSURE TEST 1 (7/17/95) RESULTS (DIRECT LOAD)								
7/17-INIT-50-1	7/17/95	11:45	0.629	2	680	62.81		
7/17-INIT-50-2	7/17/95	11:45	0.626	2	709	65.49	64.47	1.84
7/17-INIT-50-3	7/17/95	11:45	0.629	2	705	65.12		
7/17-FNL-50-1	7/17/95	14:51	0.642	2	658	60.78		
7/17-FNL-50-2	7/17/95	14:53	0.64	2	708	65.40	63.27	3.01
7/17-FNL-50-3	7/17/95	14:56	0.636	2	689	63.64		
EXPOSURE TEST 1 (7/17/95) RESULTS (INDIRECT LOAD)								
7/17-INIT-50-1D	7/17/95	11:45	0.629	2	605	55.88		
7/17-INIT-50-2D	7/17/95	11:45	0.626	2	604	55.79	56.31	1.20
7/17-INIT-50-3D	7/17/95	11:45	0.629	2	620	57.27		
EXPOSURE TEST 2 (7/24/95) RESULTS								
7/24-INIT-50-1	7/24/95	11:40	0.565	2	715	66.04		
7/24-INIT-50-2	7/24/95	11:42	0.565	2	595	54.96	49.11	34.08
7/24-INIT-50-3	7/24/95	11:43	0.568	2	285	26.32		
ANALYSIS OF TCE CALIBRATION GAS MIXTURE								
49.5 ppm STD-1	7/21/95	-	-	2	596	55.05		
49.5 ppm STD-2	7/21/95	-	-	2	578	53.39	51.36	8.00
49.5 ppm STD-3	7/21/95	-	-	2	494	45.63		
98.6 ppm STD-1	7/21/95	-	-	1	493	91.07		
98.6 ppm STD-2	7/21/95	-	-	1	562	103.82	96.74	5.48
98.6 ppm STD-3	7/21/95	-	-	1	516	95.32		
EXPOSURE TEST 3 (7/31/95) RESULTS								
7/31-INIT-100-1	7/31/95	11:58	1.041	1	579	106.96		
7/31-INIT-100-2	7/31/95	12:00	1.042	1	613	113.24	114.16	5.51
7/31-INIT-100-3	7/31/95	12:02	1.055	1	662	122.29		
EXPOSURE TEST 4 (8/7/95) RESULTS								
8/7-FNL-100-1	8/7/95	14:28	1.033	1	709	130.98		
8/7-FNL-100-2	8/7/95	14:30	1.035	1	632	116.75	116.75	9.95
8/7-FNL-100-3	8/7/95	14:33	1.027	1	555	102.53		

Calculations: ppm = [(ng / mL)/ MW] * [0.082 *(273 + C)]
MW of trichloroethylene = 131.39
C = 23
ppm = [Measured amount (ng/cart) / collected volume (mL)] * [(0.082*296) / 131.39]

Table 12. Chamber TCE concentrations based On Tenax cartridge analysis (continued).

Sample Code	Date Collected	Time Collected	MIRAN Reading (volts)	Volume Collected (mL)	Measured Amount (ng/cart)	Calc. Chamber Conc. (ppm)	Average Conc. (ppm)	%RSD
EXPOSURE TEST 5 (8/14/95) RESULTS								
8/14-INIT-100-1	8/14/95	11:03	1.01	1	655	121.00		
8/14-INIT-100-2	8/14/95	11:06	1.09	1	594	109.73	117.67	4.79
8/14-INIT-100-3	8/14/95	11:08	1.029	1	662	122.29		
EXPOSURE TEST 6 (8/21/95) RESULTS								
8/21-INIT-50-1	8/21/95	12:04	0.521	2	648	59.85		
8/21-INIT-50-2	8/21/95	12:08	0.528	2	566	52.28	56.59	5.62
8/21-INIT-50-3	8/21/95	12:12	0.534	2	624	57.64		
EXPOSURE TEST 7 (9/25/95) RESULTS								
9/25-INIT-100-1	9/25/95	12:18	1.019	1	590	108.99		
9/25-INIT-100-2	9/25/95	12:21	1.001	1	494	91.26	101.73	7.46
9/25-INIT-100-3	9/25/95	12:22	1.001	1	568	104.93		
9/25-INT-0-1	9/25/95	15:08	--	8146	3124	0.07	0.06	9.22
9/25-INT-0-2	9/25/95	15:08	--	9811	3127	0.06		
9/25-FNL-0-1	9/25/95	16:55	--	9510	2189	0.04	0.04	5.07
9/25-FNL-0-2	9/25/95	16:55	--	9212	2347	0.05		
EXPOSURE TEST 8 (10/2/95) RESULTS								
10/2-INIT-100-1	10/2/95	12:10	1.02	1	317	58.56		
10/2-INIT-100-2	10/2/95	12:13	1.02	1	311	57.45	59.98	4.71
10/2-INIT-100-3	10/2/95	12:15	1.075	1	346	63.92		
10/2-INIT-0-1	10/2/95	15:20	--	9282	1666	0.03	0.03	0.93
10/2-INIT-0-2	10/2/95	15:20	--	9603	1692	0.03		
10/2-FNL-0-1	10/2/95	17:04	--	9294	1024	0.02	0.02	2.65
10/2-FNL-0-2	10/2/95	17:04	--	9546	1109	0.02		
EXPOSURE TEST 9 (10/9/95) RESULTS								
10/9-INIT-100-1	10/9/95	13:47	1.02	1	303	55.97		
10/9-INIT-100-2	10/9/95	13:49	1.005	1	359	66.32	79.19	32.66
10/9-INIT-100-3	10/9/95	13:50	1.0065	1	624	115.27		
EXPOSURE TEST 10 (11/6/95) RESULTS								
BLANK CARTRIDGE					0			
11/6-INIT-100-1	11/6/95	13:51	1.018	1	568	104.93		
11/6-INIT-100-2	11/6/95	13:54	1.01	1	524	96.80	94.15	10.68
11/6-INIT-100-3	11/6/95	13:57	1.025	1	437	80.73		
EXPOSURE TEST 11 (11/27/95) RESULTS								
11/27-INIT-100-1	11/27/95	12:50	1.064	1	546	100.86		
11/27-INIT-100-2	11/27/95	12:52	1.036	1	470	86.82	92.60	6.29
11/27-INIT-100-3	11/27/95	12:57	0.977	1	520	96.06		
11/27-INIT-100-4	11/27/95	12:59	1.03	1	469	86.64		

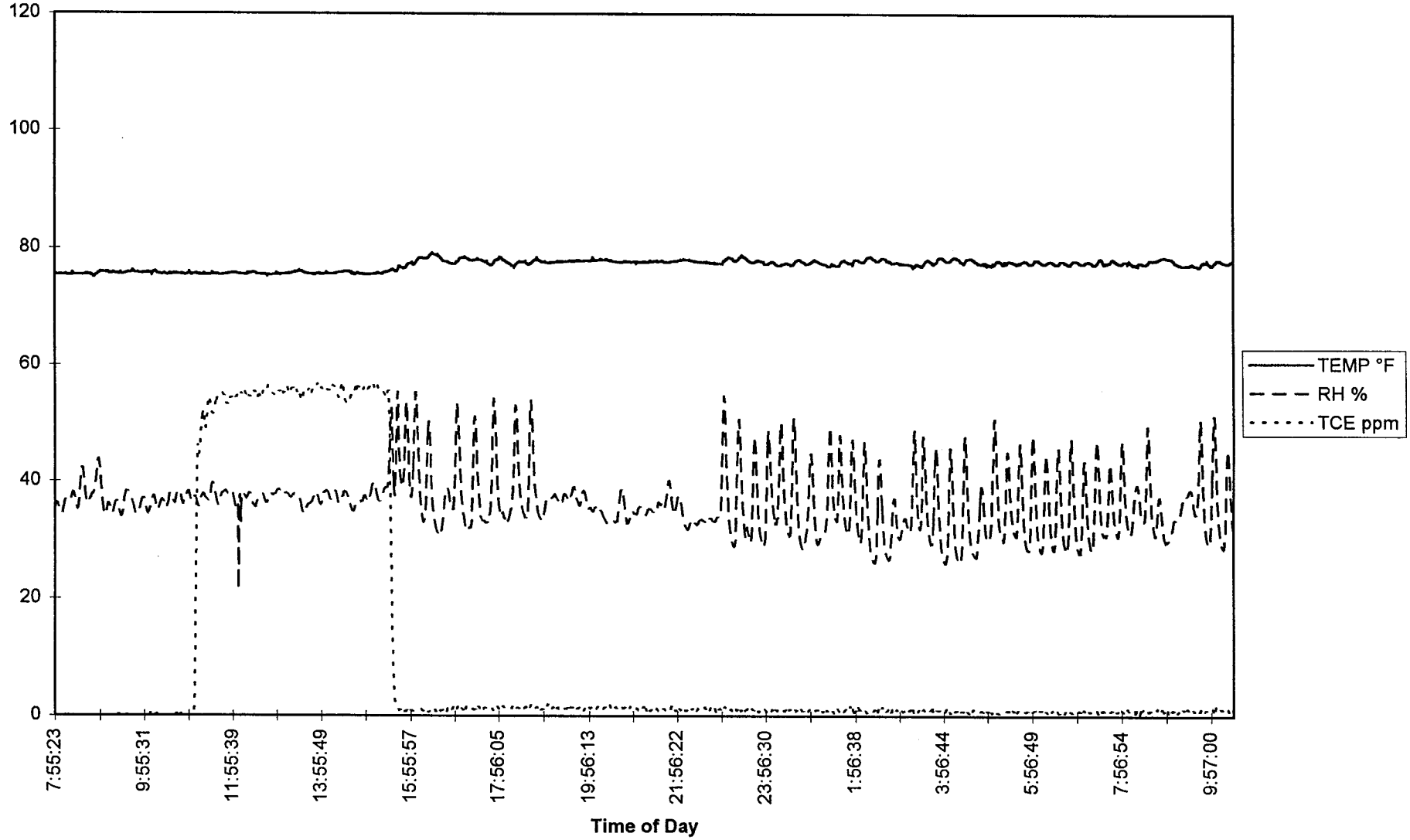
Calculations: ppm = [(ng / mL)/ MW] * [0.082 *(273 + C)]
MW of trichloroethylene = 131.39
C = 23
ppm = [Measured amount (ng/cart) / collected volume (mL)] * [(0.082*296) / 131.39]

Table 13. Data for Figure 3, comparison of Miran and GC/MS sample data.

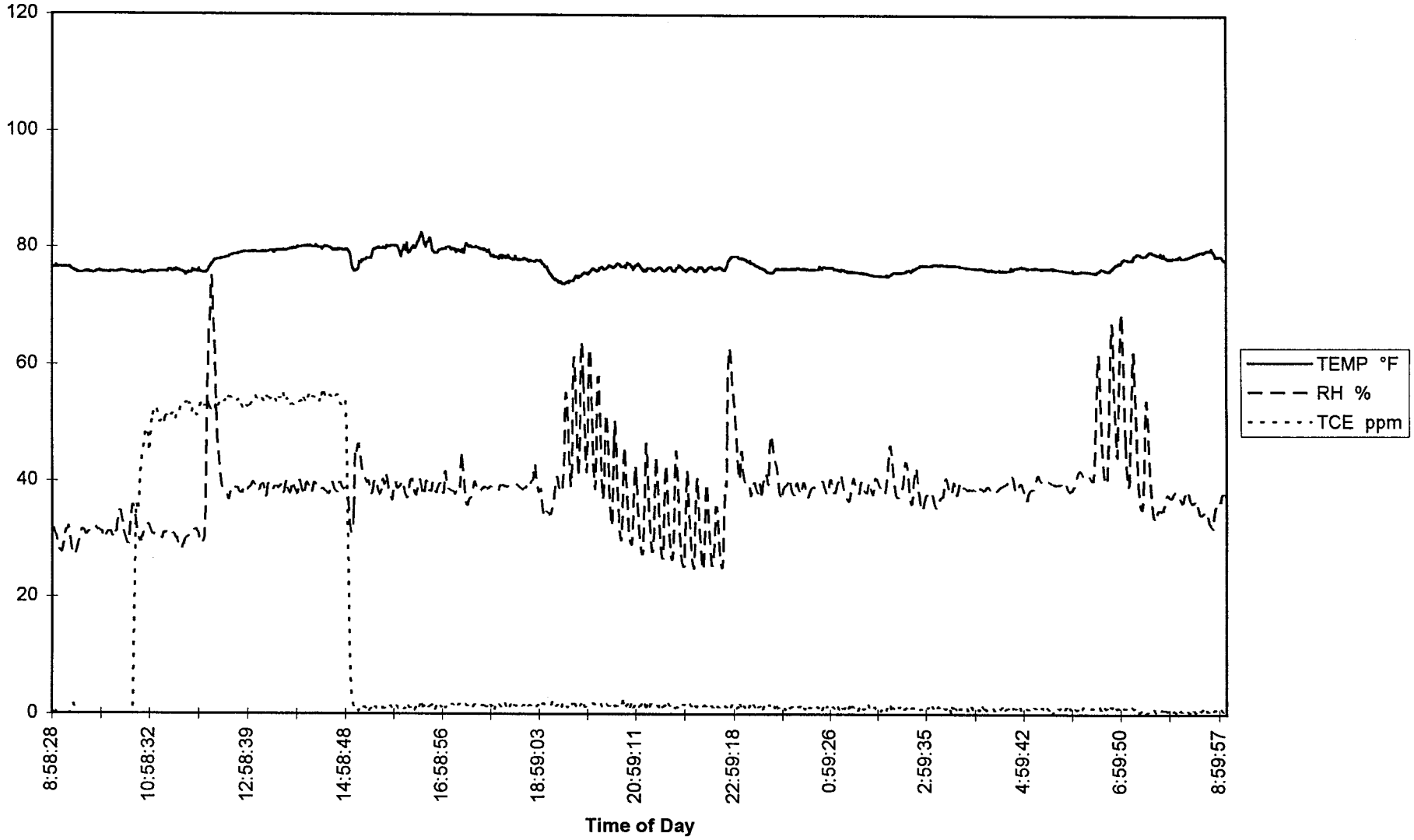
Sample #	Exper #	Time	GC/MS	Nearest 3 Miran			Mean
			ppm	ppm	ppm	ppm	ppm
7/17-INIT-50-1	1	11:45	62.81	54.71	54.62	53.33	54.22
7/17-INIT-50-2	1	11:45	65.49	54.71	54.62	53.33	54.22
7/17-INIT-50-3	1	11:45	65.12	54.71	54.62	53.33	54.22
7/17-FNL-50-1	1	11:51	60.78	53.71	54.19	54.51	54.14
7/17-FNL-50-2	1	11:53	65.4	54.51	54.29	54.50	54.43
7/17-FNL-50-3	1	11:56	53.64	54.44	54.42	55.05	54.63
7/17-INIT-50-1D	1	11:45	55.88	54.71	54.62	53.33	54.22
7/17-INIT-50-2D	1	11:45	55.79	54.71	54.62	53.33	54.22
7/17-INIT-50-3D	1	11:45	57.27	54.71	54.62	53.33	54.22
7/24-INIT-50-1	2	11:40	66.04	52.85	52.75	53.32	52.98
7/24-INIT-50-2	2	11:42	54.96	53.32	53.31	53.57	53.40
7/24-INIT-50-3	2	11:43	26.32	53.31	53.57	52.50	53.12
49.5ppm-STD-1	CAL		55.05	49.50	49.50	49.50	49.50
49.5ppm-STD-2	CAL		53.39	49.50	49.50	49.50	49.50
49.5ppm-STD-3	CAL		45.63	49.50	49.50	49.50	49.50
98.6ppm-STD-1	CAL		91.07	98.60	98.60	98.60	98.60
98.6ppm-STD-2	CAL		103.82	98.60	98.60	98.60	98.60
98.6ppm-STD-3	CAL		95.32	98.60	98.60	98.60	98.60
7/31-INIT-100-1	3	11:58	106.96	104.34	103.98	103.97	104.10
7/31-INIT-100-2	3	12:00	113.24	103.97	105.49	104.58	104.68
7/31-INIT-100-3	3	12:02	122.29	104.58	104.88	104.46	104.64
8/7-FNL-100-1	4	14:28	130.98	103.14	103.29	102.51	102.98
8/7-FNL-100-2	4	14:30	116.75	102.51	102.32	103.78	102.87
8/7-FNL-100-3	4	14:33	102.53	103.97	104.08	102.50	103.52
8/14-INIT-100-1	5	11:03	121	101.50	99.58	102.26	101.12
8/14-INIT-100-2	5	11:06	109.73	102.82	103.58	103.73	103.38
8/14-INIT-100-3	5	11:08	122.29	103.73	103.85	101.75	103.11
8/21-INIT-50-1	6	12:04	59.85	49.16	49.24	49.52	49.31
8/21-INIT-50-2	6	12:08	52.28	49.54	49.44	49.76	49.58
8/21-INIT-50-3	6	12:12	57.64	50.33	50.07	50.54	50.31
9/25-INIT-100-1	7	12:18	108.99	N/A	N/A	N/A	N/A
9/25-INIT-100-2	7	12:21	91.26	N/A	N/A	N/A	N/A
9/25-INIT-100-3	7	12:22	104.93	N/A	N/A	N/A	N/A
9/25-INT-0-1	7	15:08	0.07	N/A	N/A	N/A	N/A
9/25-INT-0-2	7	15:08	0.06	N/A	N/A	N/A	N/A
9/25-FNL-0-1	7	16:55	0.04	N/A	N/A	N/A	N/A
9/25-FNL-0-2	7	16:55	0.05	N/A	N/A	N/A	N/A
10/2-INIT-100-1	8	12:10	58.56	N/A	N/A	N/A	N/A
10/2-INIT-100-2	8	12:13	57.45	N/A	N/A	N/A	N/A
10/2-INIT-100-3	8	12:15	63.92	N/A	N/A	N/A	N/A
10/2-INIT-0-1	8	15:20	0.03	N/A	N/A	N/A	N/A
10/2-INIT-0-2	8	15:20	0.03	N/A	N/A	N/A	N/A
10/2-FNL-0-1	8	17:04	0.02	N/A	N/A	N/A	N/A
10/2-FNL-0-2	8	17:04	0.02	N/A	N/A	N/A	N/A
10/9-INIT-100-1	9	13:47	55.97	100.30	100.27	100.51	100.36
10/9-INIT-100-2	9	13:49	66.32	100.51	100.66	100.15	100.44
10/9-INIT-100-3	9	13:50	115.27	100.66	100.15	101.12	100.64
11/6-INIT-100-1	10	13:51	104.3	101.12	101.72	100.75	101.20
11/6-INIT-100-2	10	13:54	96.8	100.03	101.00	101.72	100.92
11/6-INIT-100-3	10	13:57	80.73	102.17	100.85	100.78	101.27
11/27-INIT-100-1	11	12:50	100.86	107.08	106.53	107.20	106.94
11/27-INIT-100-2	11	12:52	86.82	107.20	105.46	104.82	105.83
11/27-INIT-100-3	11	12:57	96.06	102.58	100.23	97.17	99.99
11/27-INIT-100-4	11	12:59	86.64	97.17	99.89	104.94	100.66

Chamber environmental records for experiment days.

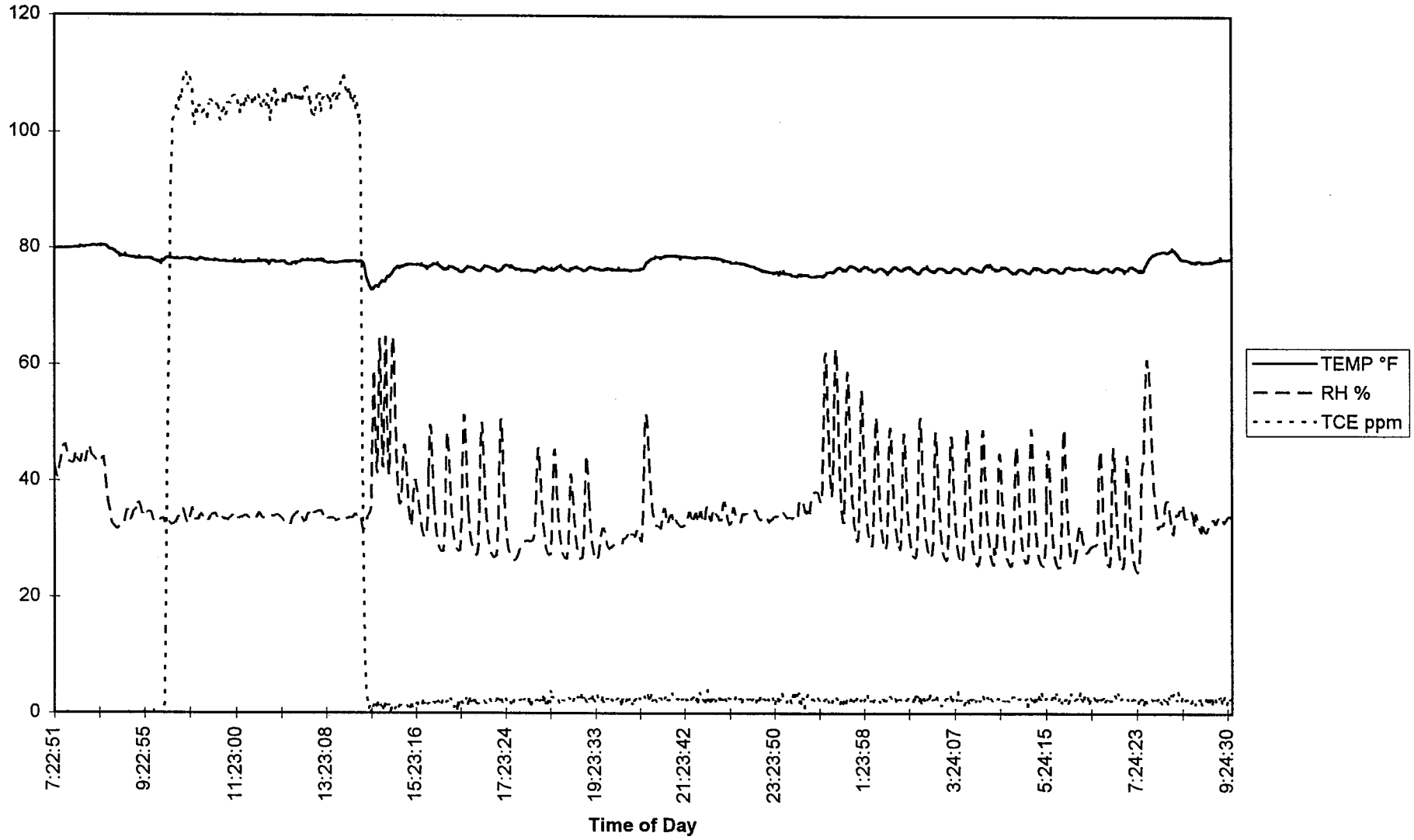
TCE Exposure: Subjects 101, 201
17 July 1995



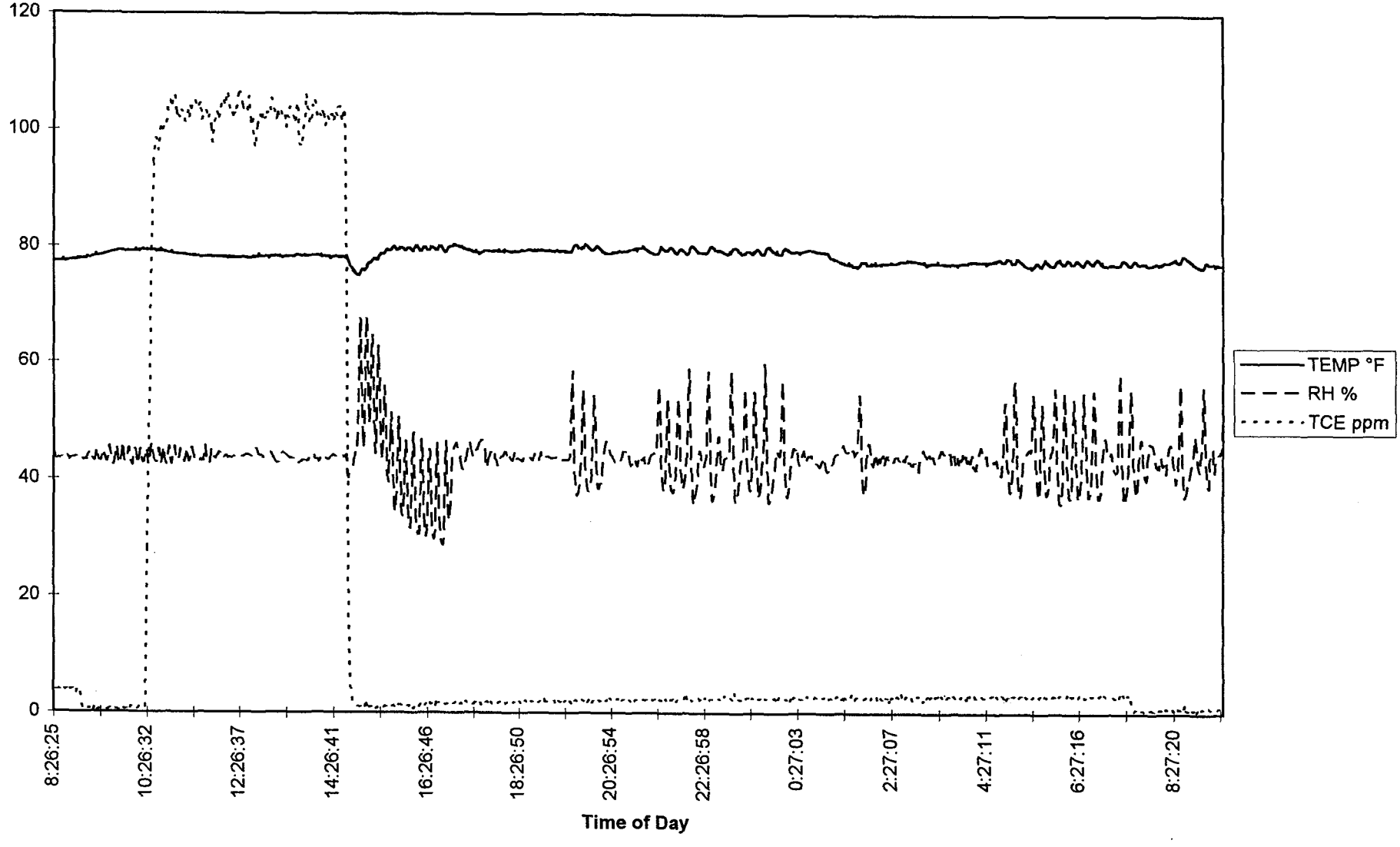
TCE Exposure: Subjects 102, 202
24 July 1995

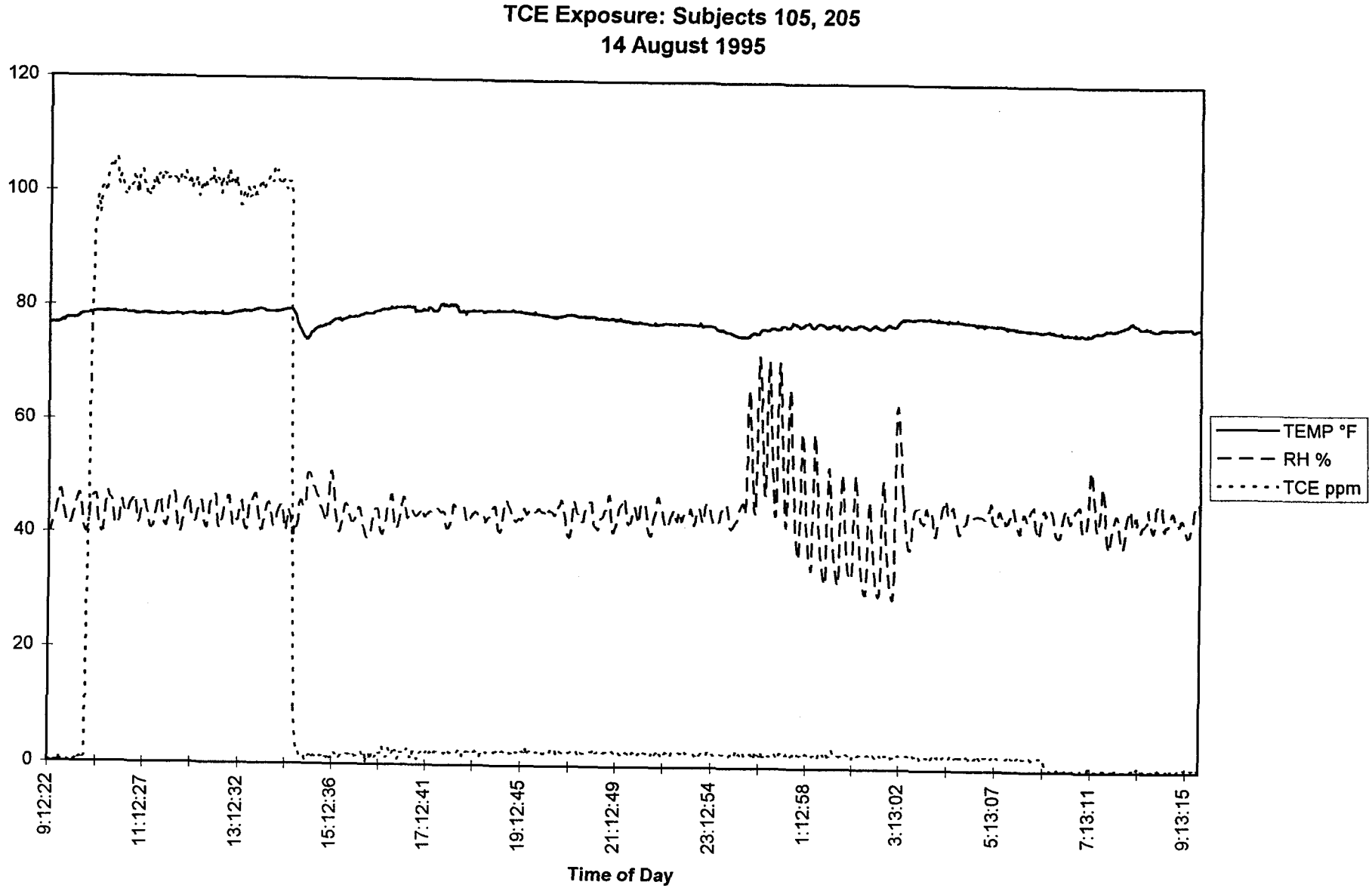


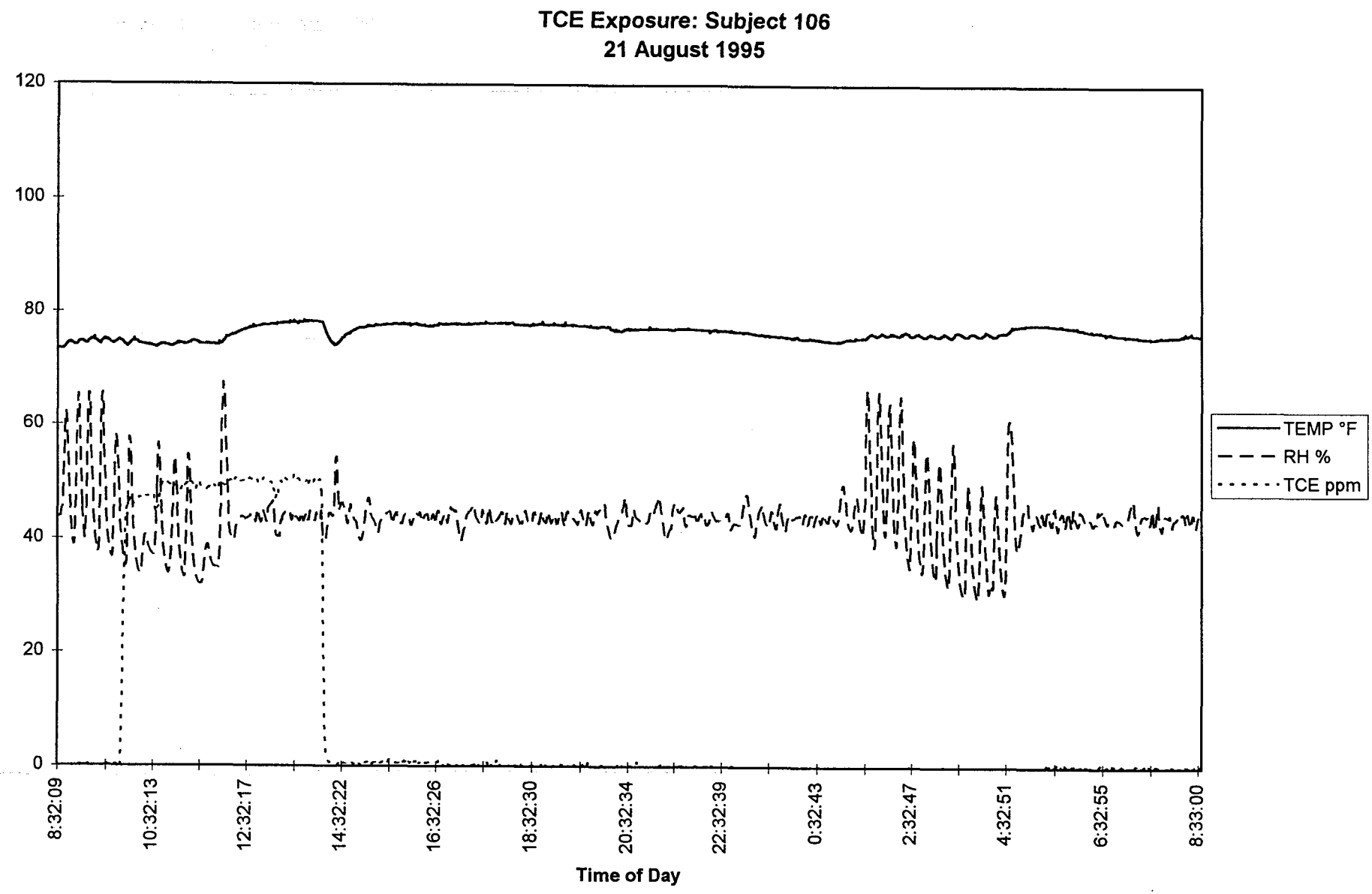
TCE Exposure: Subjects 103, 203
31 July 1995



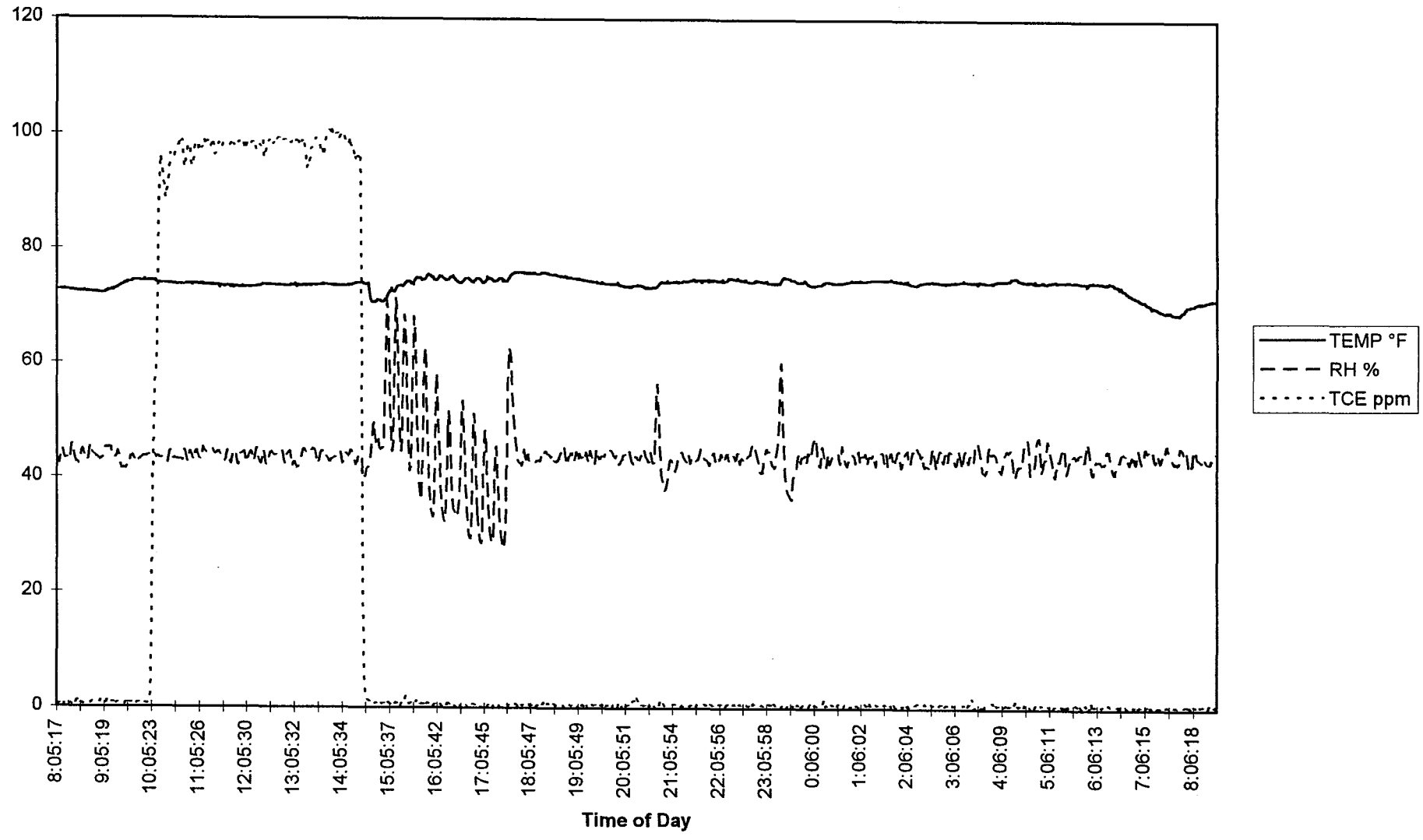
TCE Exposure: Subjects 104, 204
7 August 1995



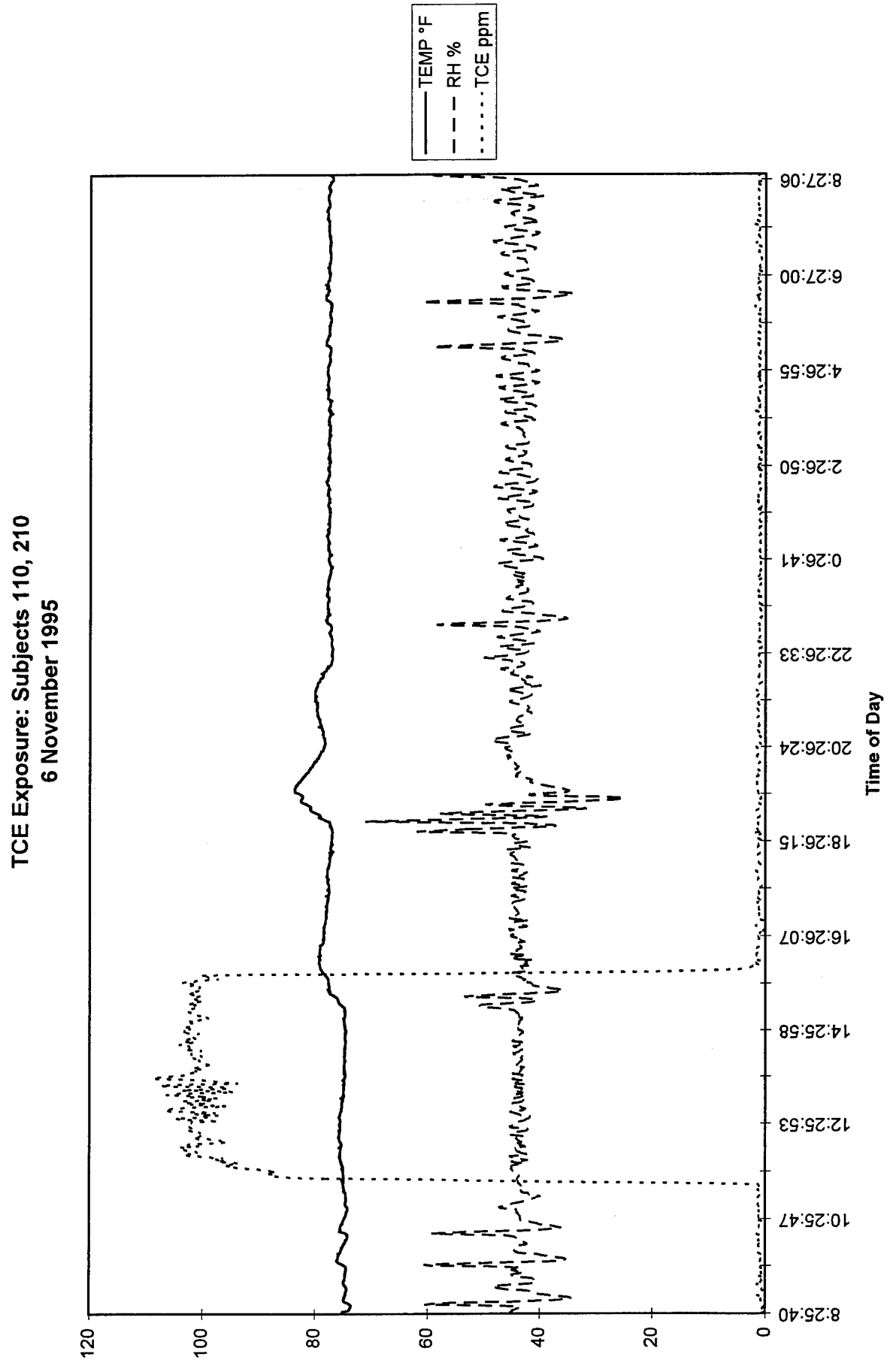

TCE Exposure: Subjects 105, 205
14 August 1995
120
100
80
60
40
20
0
9:12:22
11:12:27
13:12:32
15:12:36
17:12:41
19:12:45
21:12:49
23:12:54
1:12:58
3:13:02
5:13:07
7:13:11
9:13:15
Time of Day
TEMP °F
RH %
TCE ppm


TCE Exposure: Subject 106
21 August 1995
120
100
80
60
40
20
0
8:32:09
10:32:13
12:32:17
14:32:22
16:32:26
18:32:30
20:32:34
22:32:39
0:32:43
2:32:47
4:32:51
6:32:55
8:33:00
Time of Day
TEMP °F
RH %
TCE ppm

TCE Exposure: Subjects 109, 209
9 October 1995



TCE10.XLS
Chart1



TCE Exposure: Subjects 111, 211
27 November 1995